

Evaluation of the Health Risks
Associated with the Treatment and
Disposal of Municipal Wastewater and Sludge

Cincinnati Univ., OH

Prepared for

Health Effects Research Lab.
Cincinnati, OH

Mar 81



U.S. Department of Commerce
National Technical Information Service

NTIS

PR81-175945

EPA 600/1-80-030
March 1981

EVALUATION OF THE HEALTH RISKS ASSOCIATED WITH THE
TREATMENT AND DISPOSAL OF MUNICIPAL WASTEWATER
AND SLUDGE

by

C.S. Clark¹, H.S. Bjornson², J.W. Holland², V.J. Elia¹, V.A. Majeti¹,
C.R. Meyer¹, W.F. Balistreri³, G.L. Van Meer¹, P.S. Gartside¹,
B.L. Specker¹, C.C. Linnemann, Jr.⁴, R. Jaffa⁴, P.V. Scarpino⁵,
K. Brenner⁵, W.J. Davis-Hoover⁵, G.W. Barrett⁶,
T.S. Anderson⁶, and D.L. Alexander¹

Departments of Environmental Health¹, Surgery², Pediatrics³,
Medicine⁴ and Civil and Environmental Engineering⁵
University of Cincinnati
and
Institute of Environmental Sciences⁶
Miami University

Grant No. R805445

Project Officer

Herbert R. Pahren
Epidemiology Division
Health Effects Research Laboratory
Cincinnati, Ohio 45268

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

REPRODUCED BY
**NATIONAL TECHNICAL
INFORMATION SERVICE**
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

EPA Form 200-4 (1-80)
(PREVIOUSLY EPA FORM 241)

TYPING GUIDE SHEET

TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA-600/1-81-030	2.	3. RECIPIENT'S ACCESSION NO. PB81 17594 5
4. TITLE AND SUBTITLE Evaluation of the Health Risks Associated with the Treatment and Disposal of Municipal Wastewater and Sludge	5. REPORT DATE March 1981	
	6. PERFORMING ORGANIZATION CODE	
7. AUTHOR(S) C. S. Clark, et al.	8. PERFORMING ORGANIZATION REPORT NO.	
9. PERFORMING ORGANIZATION NAME AND ADDRESS Departments of Environmental Health, Surgery, Pediatrics, Medicine and Civil and Environmental Engineering University of Cincinnati Cincinnati, Ohio 45267	10. PROGRAM ELEMENT NO. CAYB1B	
	11. CONTRACT/GRANT NO. R805445	
12. SPONSORING AGENCY NAME AND ADDRESS U.S. Environmental Protection Agency Health Effects Research Laboratory 26 W. St. Clair St. Cincinnati, Ohio 45268	13. TYPE OF REPORT AND PERIOD COVERED Final 2/22/78 - 5/21/80	
	14. SPONSORING AGENCY CODE	
15. SUPPLEMENTARY NOTES		
16. ABSTRACT In this study, started in 1977, clinical and serologic evaluations of workers involved in composting of wastewater treatment plant sludge by the aerated pile method was initiated to evaluate the potential health effects of exposure to <i>Aspergillus fumigatus</i> and other viable and nonviable components of sludge. A health study consisting of analysis of blood specimens for liver and kidney function parameters, determination of serum and urine bile acid concentrations, physical examinations, interviews, and air and water monitoring was conducted of residents exposed to carbon tetrachloride and other toxic chemicals in drinking water as a result of contamination of domestic wells by a nearby hazardous waste dump. A study was undertaken to determine if evidence of exposure to hexachlorocyclopentadiene (HEX), hexachlorobicycloheptadiene (HEX-BCH), heptachlorobicycloheptane, and chlordene could be detected by urine analysis of workers exposed to chlorinated insecticide substances discharged to the sewer by a pesticide formulator. In order to assess the potential for health risks associated with the spray irrigation of wastewater, a clinical and virus serologic evaluation of workers and wastewater and air monitoring for viruses, pathogenic bacteria, and volatile organic chemicals was conducted. A field ecosystem study was carried out to evaluate the effects of land application of heat dried municipal wastewater treatment plant sludge (Milorganite) on vegetation, insects and meadow voles. The potential health effects associated with sludge incineration and pyrolysis are also presented.		
17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
MARGIN
AREA

DROPPED
HEAD-
ING OR
SECTION
HERE

DISCLAIMER

This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

BEGIN
LAST LINE
OF TEXT

5/8"

ii

PAGE NUMBER

BOTTOM OF
MARGIN AREA;
OUTSIDE
DIMENSION
FOR TABLE
AND ILLU-
STRATION

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

CHOPPED
HEAD
BETWEEN
SECTIONS
HERE

FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and governmental concern about the dangers of pollution to the health and welfare to the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our national environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA. To this end, HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents found in the environment. In addition to the valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

This report contains several diversified health related evaluations relating to the treatment and disposal of wastewater and sludge. Hopefully, the information will be of value to those planning, operating, or regulating such facilities so that health effects may be minimized.



R.J. Garner
Director
Health Effects Research Laboratory

BEGIN
LAST LINE
OF TEXT

RIGHT OF
IMAGE AREA
TOP
LINE OF
TEXT
HERE
PRINTED

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD,
BEGIN
SECTIONS
HERE

ABSTRACT

Information concerning the nature and extent of human health risks involved in the treatment and disposal of municipal wastewater and sludge is scarce. A major research program to fill some of the gaps has been undertaken at the University of Cincinnati, in the Department of Environmental Health, since 1975, which involved collaboration with investigators in other Departments of the University and at Miami University. The first phase of this effort focussed on occupational exposure to viruses and bacteria associated with sewer maintenance and conventional activated sludge wastewater treatment. The second phase of this effort, starting in 1977, included study of exposure to different agents: organic chemicals, fungi and endotoxins; and different processes: composting of wastewater treatment plant sludges, hazardous waste disposal, the spray irrigation of wastewater, and the land application of sludge. The various projects carried out during the second phase of the program are described in this report.

9-1/8"

A study of workers involved in composting of wastewater treatment plant sludge was initiated to evaluate the potential health effects of exposure to Aspergillus fumigatus and other viable and nonviable components of sludge in three eastern cities. The study consisted of clinical and serologic evaluation of workers five times a year, physical examinations, and environmental monitoring. The A. fumigatus spores from oropharyngeal and anterior nares cultures of compost workers were isolated with comparatively high frequency compared to control groups. Preliminary data also indicated an apparent antibody response in some of the workers to lipopolysaccharide prepared from compost samples.

A health study was conducted of the residents of a small community exposed to toxic chemicals in drinking water as a result of contamination of their domestic wells by a nearby toxic wastes dump. Testing performed in November 1978 after most use of the contaminated water had ended indicated elevated levels of the serum enzymes alkaline phosphatase, serum glutamic pyruvic transaminase, and serum glutamic oxaloacetic acid in many of the residents. Two months later many of the persons with abnormal test results had results in the normal range. Six exposed individuals had slightly enlarged livers compared to one in the intermediate group. The nonfasting serum concentrations of the bile acid sulfated conjugates of lithocholate (SLCC) were significantly lower in the exposed group than in the controls in January 1979. Air samples collected in some of the homes showed the presence of hexachlorocyclopentadiene.

BEGIN
LAST LINE
OF TEXT

Workers exposed to chlorinated insecticide intermediates discharged to the sewer by a pesticide formulator were studied at the North Wastewater

3/8"

iv

BOTTOM OF
IMAGE AREA,
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD;
BEGIN
SECTIONS
HERE

Treatment Plant, Memphis, TN. The study was undertaken to determine if evidence of exposure to toxic chemicals could be detected in the workers. The study showed that the wastewater and air samples at the North Plant contained several contaminants including hexachlorocyclopentadiene (HEX), hexachlorobicycloheptadiene (HEX-BCH), heptachlorobicycloheptene, and chlordene. A number of workers at the North and Maxson (control) plants had HEX and HEX-BCH in their urine specimens. However, urinary excretions of HEX-BCH were significantly higher in North Plant workers than in Maxson Plant workers. Also, urinary excretion of HEX-BCH was found to increase during the work shift for workers at the North Plant, whereas it decreased during the work shift at the Maxson Plant. The significance of the presence of these chemicals in the urine of the workers and the potential long-term health effects of exposure to these chemicals are not known.

The Muskegon County wastewater spray irrigation system study consisted of clinical and virus serologic evaluation of the workers and environmental monitoring for viruses, pathogenic bacteria, and volatile organic chemicals in wastewater and air samples. Although viruses were present in influent wastewater and sometimes in the storage lagoon, no animal viruses were detected either in the lagoon wastewater just prior to spray application or in the aerosol samples collected at the aeration basin. Klebsiella was found to be the most predominant of the gram-negative rod pathogens detected in air samples. The concentrations of selected volatile organic chemicals detected downwind of the spray irrigation rigs were much below the recommended occupational standards. Clinical evaluation of the workers did not show any differences in illness and virus isolation rates compared to a control group. The study showed that the antibody titers to coxsackievirus B5 were significantly higher in spray irrigation nozzle cleaners, the workers with the greatest and most direct exposure to wastewaters. No other differences in exposed and control workers were detected in levels of antibody to seven other viruses.

A field ecosystem study was carried out at Miami University, Oxford, Ohio, to evaluate the effects of land application of heat dried municipal wastewater treatment plant sludge (Milorganite) on vegetation, insects and meadow voles. The study concluded that the old-field community is more stable than the wheat field and better suited for sludge application than the wheat fields. The survivorship, longevity, percentage of breeding adults and recruitment rates of voles were not affected by sludge treatment. Application rates of Pb, Zn, Cd, and Cu were much higher in the sludge-treated fields than in those treated with a nitrogen and phosphorous-equivalent commercial fertilizer. There were no differences in tissue concentrations of Pb, Zn and Cu of meadow voles from any of the fields. However, Cd concentrations were elevated in kidneys and livers of all voles from sludge-treated plots for both annual and perennial fields. Although there were no differences in liver degeneration between voles from the sludge and commercial fertilizer field plots, a definite effect is present in the sludge and fertilizer-exposed group compared to the control group.

BEGIN
LAST LINE
OF TEXT

Reports have been prepared from an evaluation of pertinent literature on potential health effects from viable and nonviable emissions (EPA-600/1-81-006) and persistent organics (EPA-600/1-80-025) associated with waste-

BOTTOM OF
IMAGE AREA;
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

water treatment plants and land application sites. The potential health effects associated with sludge incineration and pyrolysis are presented in this report.

DROPPED
HEAD,
BEGIN
SECTIONS
HERE

This report was submitted in fulfillment of Grant No. R 805445 by the Department of Environmental Health, University of Cincinnati, under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period February 22, 1978 to May 21, 1980.

6-1/2"

9-1/8"

BEGIN
LAST LINE
OF TEXT

3/8"

vi

PAGE NUMBER

BOTTOM OF
IMAGE AREA,
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTIONS
HERE

CONTENTS

Foreword.....	iii
Abstract.....	iv
Figures.....	x
Tables.....	xii
Acknowledgments.....	xix
1. Introduction.....	1
2. Conclusions.....	5
3. Recommendations.....	12
4. Occupational Hazards Associated With Sludge Composting.....	14
Background.....	14
Environmental monitoring.....	17
Potential health effects of <u>Aspergillus fumigatus</u> ...	17
Elements of compost workers study.....	19
Population groups selected.....	19
Methods.....	21
Clinical and serological.....	21
Environmental monitoring.....	23
Results.....	24
Populations recruited.....	24
Air sampling.....	25
A. <u>fumigatus</u> in study participants.....	30
Liver function and total immunoglobulin	
determination.....	32
Delayed hypersensitivity to aspergillus antigens....	32
C-reactive protein (CRP).....	32
Eosinophil count.....	32
Chest x-ray findings.....	33
Antibody to A. <u>fumigatus</u>	33
Antibody to Tipopolysaccharide (LPS).....	33
Health effects associated with composting.....	33
Discussion and conclusion.....	34
5. An Environmental Health Survey of Drinking Water Contamination	
by Leachate from a Pesticide Waste Dump - Hardeman County,	
Tennessee.....	36
Background.....	36
Methods and procedures.....	37
Results - November 1978.....	42
Summary.....	49
Results - January 1979.....	49
Discussion and conclusions.....	64

BEGIN
LAST LINE
OF TEXT

vii

PAGE NUMBER

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSIONS
FOR TABLES
AND FIGURE
CAPTIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
MARGIN
AREA

DRIPPED
HEAD
BEGINS
SECTION
HERE

6. Evaluation of Worker Exposure to Pesticide Chemicals at a Memphis, Tennessee, Wastewater Treatment Plant.....	68
Study design.....	69
Analytical methods.....	72
Statistical methods.....	73
Results.....	73
Urine analysis and illness symptoms.....	73
Summary of results of analysis of urine data and illness symptoms.....	86
Environmental monitoring.....	86
Medical.....	88
Discussion.....	88
7. Viruses in Aerosols and Wastewater at a Wastewater Spray Irrigation System.....	94
Site description.....	94
Methods.....	96
XM2 biological sampler/collector.....	96
Collection liquids.....	96
Measurement of the air velocity of the sampler air unit.....	96
Cleaning and sterilization procedures.....	100
Sterility tests.....	100
Heathkit portable weather station, model ID-1290....	102
Sampling procedure.....	102
Sample processing and virus assay.....	106
Coliphage assay.....	107
Bacterial assay.....	107
Results and discussion.....	108
8. Bacterial Aerosol Enumeration and Identification at a Wastewater Spray Irrigation System.....	122
Materials and methods.....	122
Air sampling.....	122
Sampling sites.....	124
Enumeration and gram-negative bacteria isolation and identification.....	124
Gram-positive bacteria identification.....	124
Results.....	127
Discussion.....	135
9. Selected Organic Chemicals in Aerosols and Wastewater at a Wastewater Spray Irrigation System.....	138
Experimental methods.....	139
Sampling and analysis procedures.....	139
Results and discussion.....	140
10. Sero-epidemiologic Survey of Workers at a Wastewater Spray Irrigation System.....	148
Methods.....	148
Results.....	149
Volunteers recruited.....	149
Reported illnesses.....	149
Virus isolations.....	149
Virus serology.....	153
Summary.....	161

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
MARGIN AREA
OUTSIDE
DIMENSION
FOR TABLE
AND ILLU-
STRATIONS

11. Effects of Sewage Treatment Plant Sludge on the Structure and Function of Two Grassland Ecosystems.....	162
Methods.....	164
Land treatment.....	164
Sludge and fertilizer analyses.....	166
Ecological methods.....	166
Histopathology.....	167
Results.....	167
Metal content of sludge and fertilizer.....	167
Primary productivity and diversity.....	170
Arthropods.....	170
Pathology report on voles.....	181
Meadow vole populations.....	185
12. Potential Health Effects from Viable and Nonviable Emissions Associated with Sludge Incineration and Pyrolysis.....	191
Introduction.....	191
Incineration.....	194
Air pollution standards.....	196
Emissions from incineration.....	197
Potential health effects of incineration.....	202
Control of air pollution from incineration.....	202
Residue disposal.....	205
Pyrolysis.....	205
Air pollution from pyrolysis.....	210
Conclusions and recommendation.....	212
References.....	213
Appendix	
A. Monthly illness history.....	227

FIGURES

Number	Page
1 Location of contaminated private wells near Hardeman County toxic waste dump (designated by X).....	39
2 Location of homes of January 1979 control group in relation to dump site (designated by letter C).....	41
3 Location of Memphis wastewater treatment plants, pesticide manufacturer and Memphis Light, Gas and Water.....	70
4 Gas chromatograms of extracts of influent wastewater samples...	87
5 Muskegon County Wastewater Management System No. 1.....	95
6 XM2 sampler air unit.....	97
7 Schematic of sample gas flow through the XM2 biological sampler/collector (from the XM2 Operator's Manual).....	98
8 Cross-section of the air collar of the sampler showing the location of the air flow readings.....	99
9 Sterile trap system for introducing air into lower tubing of the sampler.....	101
10 Location of the XM2 biological sampler/collector during the sampler runs.....	103
11 Schematic of aeration basins #1 and #2 showing the location of the XM2 biological sampler/collector during the specified days of the sampling runs.....	104
12 Schematic showing the position of the XM2 biological sampler/collector in relation to the edge of the wastewater.....	105
13 Schematic diagram of a six-stage Andersen sampler.....	123
14 Map of Muskegon Wastewater Management System No. 1 showing the air sampling sites.....	125
15 Flow diagram for enumeration and gram-negative bacteria isolation and identification scheme.....	126

FIGURES (continued)

<u>Number</u>		<u>Page</u>
16	Flow diagram of the replication and identification scheme for gram-positive bacteria.....	128
17	Relative proportions of respirable bacteria identified downwind of aeration basin in 2.06 cubic meters of air.....	130
18	Relative proportions of respirable bacteria identified upwind of aeration basin in 2.01 cubic meters of air.....	131
19	Relative proportions of respirable bacteria identified downwind of field rigs in 3.97 cubic meters of air.....	132
20	Relative proportions of respirable bacteria identified upwind of field rigs in 3.97 cubic meters of air.....	133
21	Relative proportions of respirable bacteria identified at other sources in 7.46 cubic meters of air.....	134
22	Schematic arrangement of individual study plots.....	165
23	Shannon-Weaver's index in the annual wheat field.....	173
24	Shannon-Weaver's index in the perennial grass field.....	174
25	Productivity in the annual wheat field.....	179
26	Productivity in the perennial grass field.....	180
27	Hydropic degeneration of hepatic cells.....	182
28	Hydropic and fatty changes in hepatic cells.....	182
29	More severe hydropic changes in hepatic cells.....	183
30	Meadow vole population densities in the wheat field.....	186
31	Meadow vole population densities in the old-field.....	187

TABLES

Number		Page
1	Scheduling of Elements of Compost Workers Study.....	20
2	Size and Type of Treatment Facilities from Which Participants Were Recruited.....	21
3	Camden Workers: (Age, Race, Sex and Exposure).....	26
4	Beltsville Workers: (Age, Race, Sex and Exposure).....	26
5	Blue Plains Workers: (Age, Race, Sex and Exposure).....	26
6	Piscataway Workers: (Age, Race, Sex and Exposure).....	26
7	Summary of 1979 Air Monitoring for Respirable Concentrations of <i>A. fumigatus</i> and Other Thermophilic Microorganisms (Colony Forming Units Per Cubic Meter, CFU/M ³).....	27
8	Summary of 1979 Air Monitoring for Respirable Concentrations of Fecal Streptococci and Fecal Coliforms (Colony Forming Units Per Cubic Meter, CFU/M ³).....	28
9	Summary of 1979 Air Monitoring for Respirable Concentrations of Coliforms and Total Bacteria (Colony Forming Units Per Cubic Meter, CFU/M ³).....	29
10	Distributions and Analyses of Study Participants According to the Highest Value of <i>Aspergillus fumigatus</i> (AF) Colonies Observed on Cultures Taken During 1979.....	31
11	Contaminants Detected in Private Wells in Toone-Teague Area of Hardeman County, Tennessee.....	38
12	Age and Sex Profile of Hardeman County Exposed Study Group and Memphis Control Group (November 1978).....	44
13	Hepatic Profile Comparison of Hardeman County Exposed Group and Memphis Control Group (November 1978).....	45
14	Variation in Hepatic Profile With Age and Sex, Hardeman County - Memphis Comparison (November 1978).....	46

TABLES (continued)

Number		Page
15	Concentrations of Selected Organic Compounds in Air Samples (1978).....	47
16	Concentrations of Selected Organic Compounds in Water Samples (1978).....	48
17	Age and Sex Profile of Exposed, Intermediate-Exposed and Control Groups in Hardeman County Study (January 1979)...	50
18	Hepatic Profile Comparison of Hardeman County Exposed, Intermediate-Exposed and Control Group (January 1979)....	51
19	Comparison of Hepatic Profile Test Results for Exposed Participants in Both November 1978 and January 1979 Studies.....	52
20	Transformation and Significant Covariables in the Statistical Analyses of Liver Function, Bile Acid and Renal Function Test Data.....	54
21	Comparison of Mean Bile Acid Levels + Standard Error) in Fasting and Nonfasting Serum and Urine Samples Collected From Participants in January 1979 Study.....	55
22	Comparison of Serum Bile Acid Levels in November 1978 Samples With Those in Serum Samples Collected From the Same Individuals in January 1979.....	56
23	Comparison of Mean Values (+ Standard Error) of Renal Profile Test Results Between Exposed and Control Groups Participating in January 1979 Study.....	57
24	Comparison of Reported Serious Illnesses in Prior Six Months.....	58
25	Concentration of Selected Organic Compounds in Water Samples and Air Samples for Residences of Study Participants (January 1979).....	60
26	Concentration of Selected Organic Chemicals in Water and Air Samples for Residents of Study Participants (March 1979).....	62
27	Comparison of Water and Air Samples of Residences of Study Participants (January and March 1979).....	65

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

TABLES (continued)

<u>Number</u>		<u>Page</u>
28	Basic Elements of Experimental Design.....	71
29	Age Distribution of Participants in May and June 1978 Urine Screenings.....	74
30	Race and Sex Distribution of Participants in May and June 1978 Urine Screenings.....	75
31	Age Distribution of Participants in September 1978 Urine Screenings.....	76
32	Race and Sex Distribution of Participants in September 1978 Urine Screenings.....	76
33	Hexachlorobicycloheptadiene (HEX-BCH) Concentrations in Urine Samples of Memphis Wastewater Treatment Plant Employees Second Half of Work Shift, May 1978.....	77
34	Hexachlorocyclopentadiene (HEX) Concentrations in Urine Samples of Memphis Wastewater Treatment Plant Employees Second Half of Work Shift, May 1978.....	77
35	Hexachlorobicycloheptadiene (HEX-BCH) Concentrations in Urine Samples of Memphis Wastewater Treatment Plant Employees, June 1978.....	79
36	Hexachlorocyclopentadiene (HEX) Concentrations in Urine Samples of Memphis Wastewater Treatment Plant Employees, June 1978.....	79
37	Prevalence of Self-Reported Symptom During May and June Surveys.....	80
38	Average Number of Symptoms Per Worker Plant and Shift, May-June 1978.....	81
39	Results of Analyses Urine Collected From Various Groups in Memphis, Tennessee During July 1978.....	83
40	Comparison of Results of HEX-BCH Analyses of Urine Specimens During July 1978 From North Plant Workers and Sampling Crew With Those From Maxson Plant Workers and Other Control Workers.....	84
41	Urine Samples of Wastewater Treatment Plant Employees Collected at the End of Shift in September 1978.....	84

DROPPED
HEAD
BEHIND
SECTION
HEAD

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
PAGE AREA
OUTSIDE
DIMENSIONS
FOR TABLES
AND FIGURES

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
PAGE
MARK

TABLES (continued)

<u>Number</u>		<u>Page</u>
42	Prevalence of Self-Reported Symptoms During September 1978 Survey.....	85
43	Concentrations of Selected Organic Compounds in Influent Wastewater at Memphis North Treatment Plant, 1978.....	89
44	Area Air Samples Collected at the Memphis North Plant, 1978..	90
45	Personal Air Monitoring of Workers at the Memphis North Wastewater Treatment Plant.....	91
46	Virus Recovery From Muskegon Air and Water Samples.....	109
47	Virus Recovery From Muskegon Raw Influent Samples.....	110
48	Comparison of the Coliphage Recovery From Muskegon Air Samples Using the Soft Agar Overlay Method and Kott's MPN Method.....	111
49	Summary of Weather Conditions at Muskegon During Air Sampling Runs.....	113
50	Coliphage Recovery From Muskegon Water Samples Using the Soft Agar Overlay Method.....	114
51	Coliphage Recovery From Muskegon Foam Samples Using the Soft Agar Overlay Method.....	115
52	Recovery of Bacteria From Muskegon Air Samples.....	117
53	Standard Plate Counts of Muskegon Water Samples.....	118
54	Comparison of Standard Plate Counts on Muskegon Foam and Aeration Basin Raw Influent Samples.....	119
55	The Advantages and Disadvantages of the XM2 Biological Sampler/Collector.....	120
56	Enumeration of Respirable Colony Forming Units Recovered at Various Sites.....	129
57	Initial Air Sampling Surveys at Muskegon County Wastewater Management System.....	141
58	Daily Air Concentrations Near Aeration Basins and Associated Wastewater Concentrations.....	142

UNWRAPPED
HEAD
BEGIN
SECTIONS
HERE

BEGIN
LAST LINE
OF TEXT

SETTING
PAGE AREA
JUDITH
DIMENSION
FOR TABLE
AND ILLU-
STRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

TABLES (continued)

<u>Number</u>		<u>Page</u>
59	Daily Air Concentrations at Aeration Basins and Influent and Effluent Wastewater Concentrations of the Aeration Basins.....	143
60	Daily Concentrations of Selected Chlorinated Organics Near Spray Irrigation Rigs.....	144
61	Air Concentrations of Selected Chlorinated Organics at Various Locations.....	146
62	Air Samples Collected at a 30 Million Gallons Per Day Primary Wastewater Treatment Plant.....	146
63	Air Concentrations of Selected Organics Collected at Various Locations at a 300 Million Gallons Per Day Activated Sludge Wastewater Treatment Plant.....	147
64	Age, Race, Sex and Length of Time on the Job for Spray Irrigation System and Road Commission Study Participants...	150
65	Age, Race, Sex and Length of Time on the Job for Study Participants Divided According to Exposure Category.....	151
66	Comparison of Illness for Spray Irrigation and Road Commission Workers by Illness Type, Worker Group, and Exposure Categories.....	152
67	Comparison of Prevalence of Antibody at Titer Level 2 or Greater for All Spray Irrigation and Road Commission Workers During the Month of July.....	154
68	Comparison of Number of Fourfold or Greater Titer Level Increases in Spray Irrigation and Road Commission Workers Using Results From First and Last Serum Collected From Each Participant.....	155
69	Comparison of Hepatitis A Antibody Tests for Spray Irrigation and Road Commission Workers at Both Age 30 and Age 40.....	156
70	Comparison of Prevalence of Antibody at Titer Level 2 or Greater for High Exposure Spray Irrigation Workers and Indoor Road Commission Workers During Month of June.....	158
71	Comparison of Number of Fourfold or Greater Titer Level Increases in High Exposure Spray Irrigation Workers and Indoor Road Commission Workers Using Results From First and Last Serum Collected From Each Participant.....	159

CHOPPED
HEAD
SEALS
SECTION
HERE

BEGIN
LAST LINE
OF TEXT

SECTION 14
IMAGE AREA
OUTSIDE
DIMENSIONAL
FOR TABLES
NOT ALLOWED

TABLES (continued)

<u>Number</u>		<u>Page</u>
72	Comparison of June Cocksackie B5 Titer Levels for Nozzle Cleaners With Those of Various Other Groups of Study Participants.....	160
73	Attributes of Three Stages of Ecosystem Development Rated According to Their Expected Capacity for Processing Sewage Sludge.....	163
74	Metal Content of Sludge, 1978 and 1979.....	168
75	Metal Content of Fertilizer, 1978 and 1979.....	169
76	Amounts of the Heavy Metals Applied to the Sludge and Fertilizer Treated Plots in 1978 and 1979 (Kilograms).....	171
77	Shannon Weaver's Diversity Index, Margelef's Richness Index, and Pielou's Evenness Index in the Annual and Perennial Field Based on Peak Biomass of Each Species.....	172
78	Peak Biomass, Litter, Standing Dead and Daily Productivity in the Annual and Perennial Field.....	175
79	Peak Biomass of Species With a Peak Greater than 5 g m ⁻² in the Annual Field.....	176
80	Peak Biomass of Species With Peak Greater Than 5 g m ⁻² in the Perennial Field.....	177
81	Mean $\pm$ S.E. for Grain Production in the Wheat Field.....	178
82	Experimental Groups of Voles and Their Exposure.....	181
83	Liver Degeneration in Voles on Agricultural Fields.....	184
84	Liver Degeneration in Voles on Fallow Fields.....	184
85	Cadmium Concentrations in Voles From the Wheat Field (1978) and First-Year Old-Field (1979) (μ g/g Wet Weight).....	188
86	Cadmium Concentrations in Voles From the Fourth-Year (1978) and Fifth-Year (1979) Old-Fields (μ g/g Wet Weight).....	189
87	Characteristics and Potential Impacts of Thermal Reduction Methods.....	192
88	Analysis of Trace Elements in Municipal Sludges From 10 U.S. Cities.....	195

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

TABLES (continued)

<u>Number</u>		<u>Page</u>
89	Emission Factors for Sewage Sludge Incinerators.....	198
90	Health Effects of Air Pollutants.....	203
91	Solid and Liquid Waste Classification.....	206
92	Classification of Waste Disposal Sites.....	207
93	Trace Metal Concentrations in Product Gas Combustion Products.....	211

DROPPED
HEAD-
BEGIN
SECTION
HERE

BOTTOM OF
IMAGE AREA
DOTTED
DIMENSIONS
FOR TABLE
HEADINGS
RELATIVE

BEGIN
LAST LINE
OF TEXT

xviii

EPA Form 2250-4 (8-80)

PRINTED BY THE U.S. GOVERNMENT

FORM NO. 2250-4

TYPING GUIDE SHEET

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

CHANGED
HEAD-
ING
SECTION
NOTE

ACKNOWLEDGMENTS

Volunteers for the compost workers study were recruited in Camden, N.J., from the Camden County Municipal Utilities Authority, Mr. John G. Stroka, Chief Engineer and Ms. Joanne D. Ochs, Compost Superintendent; in Beltsville, MD, from the operator of the compost facility, the Maryland Environmental Services, Mr. Clinton R. Albrecht, Chief, Design and Construction and Mr. Charles Schriver, Compost Superintendent; in Washington, D.C., from the District of Columbia Department of Environmental Services, Dr. Bailus Walker, Jr., former Environmental Health Scientist-Administrator, Mr. John Thomas, Superintendent Blue Plains Water Pollution Control Plant and the TYROC Waste Management, Inc., Mr. James A. Madison, Executive Vice President, former operator of compost operations at the Blue Plains Plant and in Piscataway, MD, from the Washington Suburban Sanitary Commission, Mr. Richard Hocesvar, Director of Maintenance and Operations and Mr. Chris Bratina, Superintendent Piscataway Water Pollution Control Plant. The cooperation of the above persons, numerous others associated with their organizations and the volunteers themselves, is sincerely appreciated. The cooperation of Dr. Paul Marsh, Dr. Pat Millner and Mr. George Wilson of the U.S. Department of Agriculture, Beltsville, MD, is also appreciated.

The Hardeman County toxic waste dump study and the Memphis water pollution control plant study were ably supported by Ms. Virginia Boyle, R.N. and Ms. Sandra Russell, R.N. of Memphis, TN, who were involved in volunteer recruitment, specimen collection and many other activities. Ms. Kathy Hunninen, M.S. of the TN Poison Control Center helped in the environmental monitoring activities. The support of the Region IV Atlanta, GA, Office of the U.S. Environmental Protection Agency and in particular, Ms. Kitty Taimi, former Environmental Engineer, is acknowledged. Drs. Larry Lowry and Mitchell Singal and Mr. Anthony Smallwood of the National Institute for Occupational Safety and Health, Cincinnati, Ohio, provided valuable guidance for these two studies. Workers for the Memphis study were recruited from the Memphis Department of Public Works, Mr. Joe Taylor, Superintendent and Mr. Tom Anderson, former Superintendent, Memphis North Plant, Mr. Maynard Stiles, Director and from the Memphis Light, Gas and Water Division, Mr. W. R. Gardiner and Mr. Bill Taylor. The support of the volunteers who participated in these studies is appreciated.

Volunteers from the Muskegon County spray irrigation exposure study were recruited from the Muskegon County Wastewater Management System, Dr. Y. W. Demirjian, Director and from the Muskegon County Road Commission, Mr. Chuck Gillan, Superintendent.

The capable efforts of Mr. John D. Sedlacek and Mr. Warren G. Taylor, formerly graduate students at the Miami University, in various aspects of the ecosystem study is acknowledged.

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

Special appreciation is expressed to my secretary, Ms. Jane Onslow, for her very capable support throughout the research described in this report.

DROPPED
HEAD:
BEGIN
SECTIONS
HERE

6-1/2"

9-1/8"

BEGIN
LAST LINE
OF TEXT

3/8"

xx

PAGE NUMBER

BOTTOM OF
IMAGE AREA;
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD;
BEGIN
SECTIONS
HERE

SECTION 1

INTRODUCTION

Contact with wastewater, wastewater sludge, and waters receiving such discharges have long been regarded as a public health risk. Assumptions concerning these risks have served as a basis for the promulgation of standards for recreational water quality and for the construction and operation of facilities for wastewater and wastewater sludge treatment. However, there is a paucity of documented evidence concerning the nature and extent of the risks involved (1). The increased pace of construction of new wastewater and sludge treatment facilities in recent years, the improvement of existing facilities and the emphasis on the land application of the residuals produced, lend impetus to the need to evaluate the risks involved. One approach is to investigate the health conditions of individuals engaged in wastewater and wastewater sludge treatment and disposal activities.

Since 1975 a major research program has been underway to evaluate the health risks involved. This research has been centered in the Department of Environmental Health but has involved extensive collaboration with investigators elsewhere in the University of Cincinnati and at Miami University. The initial effort focussed on exposure to viral and bacterial hazards associated with sewer maintenance and conventional activated sludge wastewater treatment (2). Beginning in 1977 this effort was expanded to include exposures to different substances: organic chemicals, fungi and endotoxins; and different processes: composting of wastewater treatment plant sludges, hazardous waste disposal and the spray irrigation of wastewater.

Sewage sludge composting by the windrow method has been practiced for a number of years by the Los Angeles County Sanitation District. More recently the development of the aerated pile composting method by the U.S. Department of Agriculture, Agricultural Research Station at Beltsville, MD, has resulted in considerable expansion of the use of sludge composting. Currently, Philadelphia, PA, Camden, N.J., Washington, D.C., Windsor, Ontario, Bangor and Portland, ME, as well as other smaller cities are engaged in municipal sludge composting. Many other cities, including New York City, are actively considering this method. A number of factors are apparently responsible for the expansion in the use of sludge composting. Among them are the prohibition of ocean dumping of sludge after 1981, the increase in the amount of sludge being produced nationally and the growing emphasis on the land application of wastewater and wastewater sludges. Municipal wastewater contains a variety of pathogenic microorganisms, many of which are concentrated in the sludge that is produced. Composting is a thermophilic process and the heat generated

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA,
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

3/8"

1

PAGE NUMBER

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD;
BEGIN
SECTIONS
HERE

helps inactivate the microorganisms present in sludge. However, the heat generated during composting also encourages proliferation of thermophilic fungi, in particular, Aspergillus fumigatus, and actinomycetes. Aspergillus fumigatus has been shown to be one of the most prevalent fungi during the sludge composting process (3). Aspergillus fumigatus is an opportunistic pathogen capable of infecting individuals whose defenses have been weakened. Aspergillus fumigatus can cause severe allergic (asthmatic) reactions in atopic individuals and aspergillosis and invasive aspergillosis in immunosuppressed patients. The workers involved in the composting process would be exposed to potential health risks from exposure to A. fumigatus and other viable components of compost. Because they are heat resistant, bacterial endotoxins are also present in composted sludge. A prospective health study was initiated in 1979 to determine if workers at municipal sludge composting facilities are subjected to an increased health risk from exposure to viable fungal particles, actinomycetes, and endotoxins in addition to the pathogenic microorganisms that are normally present in municipal wastewater and sludge. A description of this study, performed under the direction of H.S. Bjornson, M.D., Ph.D., of the Department of Surgery, and a preliminary assessment of the results obtained in 1979 are presented in Section 4: Occupational Hazards Associated with Sludge Composting.

Improper disposal of hazardous chemical waste is increasingly being recognized as a source of potential human health hazard. A health study of the residents of a small community exposed to toxic chemicals in drinking water as a result of contamination of their domestic wells was initiated in 1978. The community under consideration is in Hardeman County, TN. Residents of this community began noticing unpleasant chemical tastes and odors from their water. Preliminary environmental monitoring indicated that the residents have been exposed to carbon tetrachloride (CCl₄) and other chlorinated organic contaminants from their private water supply wells and possibly in ambient air. The source of these contaminants in groundwater is most probably from leachates from a chemical waste landfill formerly used by a Memphis, TN, pesticide formulator which manufactures chlorinated insecticides. In November 1978, the residents of the affected community were advised by U.S. Environmental Protection Agency (EPA), Region IV, Atlanta office, to stop using the water because monitoring by EPA showed high concentrations of CCl₄ (up to 10,000 µg/L) in the water samples. A study to determine if the residents of the Toone-Teague community, Hardeman County, TN, showed any detectable health effects from exposure to toxic organic chemicals in their drinking water was conducted during the Fall and Winter of 1978-1979. W. F. Balistreri, M.D., of the Department of Pediatrics, assisted members of the Department of Environmental Health with this study. Section 5: An Environmental Health Survey of Drinking Water Contamination by Leachate from a Pesticide Waste Dump - Hardeman County, Tennessee, is a description of this study and its results.

During the prospective seroepidemiological study of the bacterial and viral disease risks associated with municipal wastewater treatment (2), an opportunity developed to investigate exposure to toxic organic chemicals emitted from the wastewater during the treatment process at one of the plants in the study. Workers at this plant, the Memphis North Wastewater Treatment Plant, had been complaining in early 1978 of acute symptoms of respiratory

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD:
BEGIN
SECTIONS
HERE

distress that they associated with periods of intense chemical odor at the plant. Other symptoms noted were dizziness, headache, and irritation of the eyes, nose, throat, lungs and skin. The treatment plant is located near a manufacturer that produces and utilizes several chlorinated organic intermediates for the formulation of flame retardants and chlorinated insecticides (notably isodrin, endrin, chlordane and heptachlor). Chemical waste from this manufacturer is discharged into a sewer that flows to the Memphis North Wastewater Treatment Plant. During 1978 exposure to several chlorinated organic compounds at this treatment plant was assessed by evaluating urinary excretion, illness symptoms and air and wastewater. A description of this study is presented in Section 6: Evaluation of Worker Exposure to Pesticide Chemicals at a Memphis, Tennessee, Wastewater Treatment Plant.

As a result of legislative actions, such as the 1972 Clean Water Act and its 1977 Amendments, land application of municipal wastewater and sludge is becoming increasingly popular as an alternative means to the more conventionally used disposal methods, such as ocean and surface water dumping, and incineration. Land application represents a recycling process in which water and plant nutrients are returned to the soil. However, a large variety of potential disease-causing microorganisms are present in municipal wastewater and conventional wastewater treatment does not completely remove the microorganisms. Spray or sprinkler application is one of the commonly used methods of land application of wastewater, but spray application also promotes aerosol formation. The volatile organic chemicals and microorganisms present in wastewater may become airborne during spray application. Aerosolization of volatile organic chemicals and microorganisms is also favored near aeration basins of the activated sludge treatment units and trickling filters at wastewater treatment plants. The workers involved with the application of the wastewater and sludge may be exposed to microorganisms and volatile organic chemicals in aerosols. In order to assess the potential health risks of workers from exposure to volatile organic chemicals and viruses in aerosols, the ambient air environment and the workers at the Muskegon County Wastewater Management System, Michigan, were evaluated. This evaluation is described in the following four sections of this report: Section 7 (Viruses in Aerosols), Section 8 (Bacterial Aerosols), Section 9 (Organic Chemical Aerosols) and Section 10 (Virus Serology Survey of Workers). Sections 7 and 8 were performed under the direction of P. S. Scarpino, Ph.D., of the Department of Civil and Environmental Engineering, and Section 10 under the direction of C. C. Linnemann, Jr., M.D., of the Department of Internal Medicine.

To date most studies of the potential harmful effects of land application of wastewater and sludge have been concerned with uptake and accumulation of heavy metals by food crops or forage plants (4,5). Some laboratory feeding experiments have demonstrated uptake and accumulation of heavy metals by consumption of sludge (6,7). It has not been adequately shown in field studies whether animals resident on sludge-treated land concentrate heavy metals in their tissue and whether the animals are adversely affected by sludge. A field ecosystem study was carried out at Miami University, Oxford, OH, under the direction of G. Barrett, Ph.D., Institute of Environmental Sciences, to evaluate the effects of land application of municipal sludge on vegetation, insects and meadow voles (Section 11).

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA,
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

3/8"

3

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD;
BEGIN
SECTIONS
HERE

Reports have been prepared from an evaluation of pertinent literature on potential health effects from viable and nonviable emissions (EPA-600/-1-81-006) and persistent organics (EPA-600/1-80-025) associated with wastewater treatment plants and land application sites. The potential health effects associated with sludge incineration and pyrolysis are presented in this report (Section 12).

6-1/2"

9-1/8"

BEGIN
LAST LINE
OF TEXT

3/8"

4

BOTTOM OF
IMAGE AREA,
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD;
BEGIN
SECTIONS
HERE

SECTION 2

CONCLUSIONS

Occupational Hazards Associated With Sludge Composting

1. Aspergillus fumigatus concentrations in air at the Piscataway Plant and at areas of the Blue Plains Plant remote from the compost pit were clearly lower than levels reached at the Beltsville and Blue Plains composting sites. Overall, about six percent of the A. fumigatus colonies were found on Andersen stage six plates representing samples thought to be small enough to reach the alveoli.
2. Fecal streptococci were detected in air samples at each of the areas where they were measured and were generally higher at compost sites.
3. Results of the environmental monitoring demonstrated that workers at each of the compost sites were exposed to markedly elevated numbers of A. fumigatus spores. This observation was further supported by the comparatively high frequency of isolation of A. fumigatus from oropharyngeal and anterior nares cultures of compost workers when compared to the intermediate-exposed and control groups.
4. Preliminary analyses of the highest results obtained for each participant on tests for the enzymes serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), measures of liver function, and levels of total IgG, IgE, and IgM, did not reveal any differences among exposure groups at any of the sites.
5. Preliminary analysis of the highest C-reactive protein (CRP) value for each individual was performed on two groupings of the data: (1) ≤ 1 mg% and > 1 mg% and (2) positive and nonreactive. The only significant difference was found among Beltsville workers. Four of nine compost workers had positive CRP values compared to none of eight intermediate-exposed workers.
6. Preliminary analyses of the highest absolute eosinophil count for each of the participants did not reveal any differences among the exposure groups at the various sites, separately or combined.
7. Chest X-rays were obtained from 74 participants and reviewed by a radiologist at the University of Cincinnati. Of these, one compost worker's X-ray exhibited an abnormality compatible with an occupationally-related disorder.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA,
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

3/8"

5

PAGE NUMBER

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD,
BEGIN
SECTIONS
HERE

8. Specific IgG directed towards A. fumigatus, as determined by the enzyme-linked immunosorbent assay (ELISA), was compared by exposure group for each site separately. At no site did the compost workers exhibit statistically higher values.
9. Titer levels of antibody directed against lipopolysaccharide (LPS) prepared from site-specific compost were not found to be higher among Camden compost workers than in control workers. However, in Beltsville and Blue Plains antibody titers among compost workers were higher than in their corresponding lower exposure groups.
10. One worker in the study, employed at a compost site, was found to have a local infection involving his right ear. This infection, caused by A. niger gradually progressed over a period of several months. Despite treatment, there was erosion of 70% of the right tympanic membrane.

An Environmental Health Survey of Drinking Water Contamination by Leachate From a Pesticide Waste Dump - Hardeman County, Tennessee

11. The residents of Toone-Teague Road in Hardeman County, TN, were exposed to carbon tetrachloride and other chlorinated organic chemicals in their drinking water and possibly in ambient air.
12. The concentrations of carbon tetrachloride, a suspected carcinogen, found in the contaminated well water (up to 18,700 $\mu\text{g/L}$) were far above the level of 2.6 $\mu\text{g/L}$ that has been proposed by the U.S. Environmental Protection Agency as a safe level for water.
13. Concentrations of carbon tetrachloride in the contaminated wells were of the order of 5000 $\mu\text{g/L}$. Assuming a consumption of one liter per day, the individuals using the contaminated water had a dose of carbon tetrachloride of about 5 mg/day.
14. There is a possibility of future contamination of the much deeper artesian aquifer, which is used at another location by Memphis, TN.
15. Air samples collected in some of the Hardeman County homes showed the presence of HEX, carbon tetrachloride and tetrachloroethylene.
16. Results from the initial screening in November 1978 revealed that the concentrations of the serum enzymes alkaline phosphatase SGPT and SGOT were elevated in the group of residents who had previously consumed the contaminated water when compared to the control group. The differences were statistically significant ($p \leq 0.05$) and appear to be associated with the ingestion of well water contaminated by leachate from a toxic waste landfill. Two months later many of the persons with abnormal test results had results in the normal range.
17. Analysis of urine samples for selected organic compounds (HEX, HEX-BCH, heptachlorobicycloheptene, chlordene and heptachlor) did not show the presence of any of these chemicals in the exposed group.

BOTTOM OF
IMAGE AREA,
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

BEGIN
LAST LINE
OF TEXT

3/8"

6

PAGE NUMBER

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD.
BEGIN
SECTIONS
HERE

18. No evidence was found for carbon tetrachloride in the urine specimens of a select group of individuals judged to have relatively high potential for exposure to carbon tetrachloride.
19. During the January 1979 survey, six individuals from the exposed group and one from the intermediate-exposed were found to have enlarged livers.
20. The mean nonfasting serum bile acid, sulfated conjugates of lithocholate (SLCC), was significantly lower in the exposed groups than in the control.
21. For those persons in the exposed group who were tested in both November 1978 and January 1979 nonfasting serum concentrations of SLCC and cholyglycine (CG) were significantly higher in November 1978 than in January 1979.
22. The residents of Toone-Teague Road, of Hardeman County, TN, have experienced acute symptoms such as skin and eye irritation, weakness in the upper and lower extremities, upper respiratory infection, shortness of breath and severe gastrointestinal symptoms including nausea. These symptoms usually subsided once the consumption of the contaminated water was stopped.
23. The potential long-term health effects of exposure to these chemicals in drinking water is not known.

Evaluation of Worker Exposure to Pesticide Chemicals at Memphis, Tennessee, Wastewater Treatment Plant

24. Influent wastewater from the North Plant, and air samples collected at the wet well and grit chamber of the North Plant, contained several contaminants including HEX, HEX-BCH, heptachlorobicycloheptene and chlordene.
25. Influent wastewater and air samples obtained at the grit chamber of the Maxson Plant, the control plant, did not contain the type of substances found in the samples obtained at the North Plant.
26. Many workers (North and Maxson Plant) had HEX or HEX-BCH in their urine specimens in May and/or June 1978. However, urinary excretions of HEX-BCH were significantly higher in North Plant workers than in Maxson Plant workers in both May and June 1978.
27. The presence of the organic chemicals in the urine samples of Maxson Plant workers may possibly be due to (a) intermittent discharge into the sewer system by either a user of these chemicals or a waste hauler, (b) discharge from a buried dump site containing these chemicals, or (c) ambient air pollution in the Memphis area.
28. Urinary excretion of HEX-BCH was found to increase during the work shift for workers at the North Plant in the June 1978 survey, whereas it decreased during the work shift at the Maxson Plant. The percent of

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA,
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTIONS
HERE

North Plant urine samples with HEX-BCH concentrations greater than 3 $\mu\text{g/L}$ increased from 7 to 41 during the work shift while in Maxson Plant samples the percent decreased from 9 to 0, during the June 1978 screening.

29. The significance of the presence of these chlorinated organic chemicals in the urine of workers is not known.

30. The workers at the North Plant experienced acute symptomatology potentially related to the toxic agents in the influent. The symptoms decreased or disappeared when the workers were removed from the contaminated environment.

31. The potential long-term health effects of exposure to these chemicals is not known.

Viruses in Aerosols and Wastewater at a Wastewater Spray Irrigation System

32. No animal viruses were detected in air samples collected at the Muskegon County Wastewater Management System using the Army prototype XM2 Biological Sampler/Collector.

33. Animal viruses were detected in raw influent samples but decreased in concentrations as the wastewater was aerated and stored in the lagoons. None were detected in wastewater at the pump station just prior to distribution to the spray irrigation rigs.

34. Using the centrifugation-filtration method all raw influent samples were found to contain viruses, ranging from 50 to 400 plaque-forming units/liter.

35. Coliphage were removed from air samples at the aeration basins in concentrations ranging from 0-9 per cubic meter of air. *E. coli* 13706 coliphage were recovered more often than the other two tested, 15597 and 11303.

36. Kott's MPN method was found to be more sensitive and consistent in detecting low levels of coliphage in air samples than the soft agar overlay method.

37. Total bacteria concentrations at the aeration basins ranged up to 7,100 colony forming units per cubic meter of air.

Bacterial Aerosols Enumeration and Identification at a Wastewater Spray Irrigation System

38. The aeration basin was a source of the following bacteria:

Aeromonas hydrophila
Escherichia coli
Enterobacter agglomerans
other *Enterobacter* spp.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA,
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD;
BEGIN
SECTIONS
HERE

Klebsiella spp.
Pasteurella spp.
Salmonella spp.
Serratia spp.

39. The field rigs were a source of:

Aeromonas hydrophila
Enterobacter agglomerans
other Enterobacter spp.
Klebsiella spp.
Pasteurella spp.
Pseudomonas spp.
Serratia spp.
Staphylococcus aureus

40. Four of the bacteria identified are in the Class 2 group of the Center for Disease Control's "Classification of Etiologic Agents"*

Klebsiella spp.
Pasteurella spp.
Salmonella spp.
Staphylococcus aureus

(By use of API-20E system for identification 19 of the 32 Klebsiella spp. were determined to be Klebsiella pneumoniae.)

41. The quantity of bacteria in the air downwind of treatment sources at the Muskegon wastewater site contained higher total numbers of bacteria and higher percentages of gram-negative bacilli, fecal-indicator bacteria, and pathogenic bacteria than upwind air.

42. The mean respirable concentration of total airborne bacteria found one meter downwind of the aeration basin at the Muskegon wastewater site was 2800 Colony Forming Units (CFU) per m³ which was significantly higher than that found 18 meters downwind of the spray irrigation rigs (i.e., 700 CFU per m³). Concentrations one meter upwind of the aeration basin and 18 meters upwind of the field rigs were 490 CFU/m³ and 660 CFU/m³, respectively.

Selected Organic Chemicals in Aerosols and Wastewater at a Wastewater Spray Irrigation System

43. Concentrations of trichloroethane, trichloroethylene and tetrachloroethylene at the Muskegon County Spray Irrigation System at the downwind edge of the aeration basins ranged up to 90 µg/L, 73 µg/L and 46 µg/L, respectively. Adjacent to the spray irrigation rigs the highest concentrations reached for the same compounds were 2.7, 9.3 and 8.6, respectively. All of these concentrations were well below the 8-hour

*Class 2 agents are those of ordinary potential hazard, and includes agents which may produce disease of varying degrees of severity.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA;
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD:
BEGIN
SECTIONS
HERE

occupational standards of 45,000 $\mu\text{g/L}$, 535,000 $\mu\text{g/L}$ and 670,000 $\mu\text{g/L}$, respectively.

44. Chloroform was not detected in the air adjacent to the spray irrigation rigs but was detected downwind of the aeration basins at concentrations ranging up to 202 $\mu\text{g/m}^3$ (8-hour occupational limit 50,000 $\mu\text{g/m}^3$). In the scrubber house chloroform was detected at 3380 $\mu\text{g/m}^3$.

45. Comparing upwind and downwind air concentrations with influent and effluent wastewater concentrations at the aeration basins showed that significant concentrations of the volatile substances measured are stripped from the wastewater during the aeration process.

Seroepidemiologic Survey of Workers at a Wastewater Spray Irrigation System

46. Illness and virus isolation rates were not significantly different in the study group of workers engaged in the spray irrigation of wastewater compared to a control group of road commission workers.

47. Antibody titers to coxsackievirus B5 were significantly higher for one subgroup of wastewater workers, the spray irrigation nozzle cleaners. This suggests that there may be a risk of viral infection only in those with the greatest and most direct exposure to wastewater.

48. The prevalence of Hepatitis A antibody correlated with age, as would be expected in normal populations, and there was no increase in the prevalence of Hepatitis A antibody in those exposed to spray irrigation.

49. Antibody titers to poliovirus 1, 2 and 3, coxsackievirus B2 and echovirus 7 and 11 were not different between the wastewater and road commission worker groups.

Effects of Sewage Treatment Plant Sludge on the Structure and Function of Two Grassland Ecosystems

50. When heat-dried Milwaukee, Wisconsin wastewater treatment plant sludge (Milorganite) and a commercial fertilizer were applied so that the amounts of added nitrogen and phosphorous were equivalent, the ratio of the amount of the following heavy metals in Milorganite to that in fertilizer was 495 for lead, 167 for cadmium, 953 for copper and 280 for zinc in 1978. In 1979 the ratios were 209, 205, 1163 and 334, respectively.

51. The Shannon-weaver diversity index was lower in the wheat fields receiving Milorganite than in the wheat fields receiving commercial fertilizer and the controls. No significant differences were observed in this index in the old grass fields suggesting that the perennial community is more stable than the annual field and better suited for application of sludge than the annual field.

BOTTOM OF
IMAGE AREA:
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

BEGIN
LAST LINE
OF TEXT

3/8"

10

PAGE NUMBER

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTIONS
HERE

52. There was a significant increase in productivity in both the perennial and annual fields in those receiving fertilizer and sludge treatments, however, the increase was larger and occurred earlier in the fertilizer-treated fields. Estimates of net primary productivity in all of the annual fields were more than 50 percent higher than those for the perennial fields. The treatment effect on plant community structure was greater in the annual field than in the perennial field, suggesting again that the perennial fields are more stable and better suited for sludge application than annual fields.
53. For arthropods the fertilizer and sludge plots exhibited consistently higher species richness values than did the control plots in both types of fields. The opposite was true with respect to apportionment values.
54. No pathologic alterations were seen in the lungs and kidneys of the meadow voles from the fertilized, sludge-treated and control plots of both the perennial or annual fields.
55. For both the annual and perennial fields the incidence of liver degeneration in the meadow voles did not differ between the fertilized and sludge treated plots but was lower in the control plots.
56. Meadow voles from the sludge-treated annual field showed a more severe liver degeneration than those from the sludge-treated perennial fields.
57. Vole population densities were affected by treatment only in the first year wheat fields where sludge treatment plots had higher densities than plots with any of the other treatments in the two years.
58. Survivorship, longevity, percentage of breeding adults and recruitment rates were not affected by sludge treatment.
59. There were no differences in tissue concentration of Pb, Zn and Cu of meadow voles from any of the fields. However, Cd concentrations were elevated in kidneys and livers of voles in both the annual and perennial sludge-treated fields.

Potential Health Effects From Viable and Nonviable Emissions Associated With Sludge Incineration and Pyrolysis

60. A literature evaluation indicated that incineration and pyrolysis processes may lead to air pollution from volatilization of heavy metals and incomplete combustion of organic chemicals, respectively. The air pollution can be controlled by the use of appropriate control measures in both incineration and pyrolysis processes and also by afterburning in case of pyrolysis.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

3/8"

11

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD;
BEGIN
SECTIONS
HERE

SECTION 3

RECOMMENDATIONS

1. Compost workers should be observed over an extended period of time to reveal abnormalities which may be related to the length of exposure.
2. The preliminary data on antibody response of the compost workers to lipopolysaccharide prepared from compost samples suggests that it may be advisable to take precautions to reduce exposure such as the use of respirators by compost workers and periodic water spraying of the compost sites to reduce dust.
3. A method should be developed to quantify the presence of endotoxin in compost and in the air.
4. Long-term medical follow-up of the residents of Toone-Teague Road, Hardeman County, TN, should be initiated.
5. Necessary precautions should be taken, if possible, to prevent the contamination of the artesian aquifer by the toxic chlorinated organic chemicals since it is used as a water supply for the City of Memphis.
6. The closure plan for the Hardeman County dump site should be designed to prevent an increased exposure, to nearby residents and to those downstream and downwind, to contaminants associated with the dump.
7. Industrial discharge of toxic chemical waste into municipal sewerage systems should be controlled and monitored.
8. A follow-up study of the Memphis workers involved should be conducted to determine if urinary excretion of HEX and HEX-BCH remains at the low levels detected in September 1978 and if any health effects are apparent.
9. The XM2 Biological Sampler/Collector should be used for a sampling period of at least several days to determine if animal viruses can be detected in aerosols sampled by this method.
10. The virus content of solid material at the bottom of the lagoons and aeration basins at the Muskegon spray irrigation site should be determined to see if future disposal of the solid material would be a problem.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

3/8"

12

PAGE NUMBER

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTIONS
HERE

11. The Kott's MPN method is recommended for use when coliphage levels are expected to be very low.
12. Because of problems with the XM2 sampler and sterilization and arcing problems in other large volume samplers, an improved large volume biological air sampler should be developed.
13. Resistant aerosolized bacteria such as Klebsiella should be explored as possible indicators of bacterial air contamination from fecal sources.
14. An examination of the serology of workers at the Muskegon spray irrigation site for antibodies to Klebsiellae should be initiated as soon as possible.
15. Routine air monitoring for selected organic compounds at the Muskegon spray irrigation site should be performed downwind of the aeration basins and in the scrubber house.
16. The observation of an apparent increased risk in nozzle cleaners at the Muskegon spray irrigation site should be confirmed. The number of available nozzle cleaners is small, but a serologic survey of former nozzle cleaners could be conducted.
17. The hypothesis that wastewater workers may be at risk of Legionnaire's disease could be evaluated further by testing for antibody to Legionella pneumophila which has been related to blue green algae. Wastewater irrigation workers may be exposed to wastewater with higher algal content than ordinary wastewater treatment plant workers.
18. For minimizing effects on small mammal populations mid-successional old-fields should be used as sites for the land application of sludge rather than early successional stages or agricultural fields.
19. Research should continue to more completely characterize the effect of sewage sludge application on mammal populations, especially to evaluate if Cd concentrations tend to increase in the kidneys and livers of animals functioning within sludge-treated plant communities.
20. Afterburning should be used in pyrolysis process to assure complete combustion of organic chemicals in sludge.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

3/8"

13

PAGE NUMBER

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD-
ING
SECTION
HERE

SECTION 4

OCCUPATIONAL HAZARDS ASSOCIATED WITH SLUDGE COMPOSTING

Workers engaged in the handling of municipal wastewater treatment plant sludge are exposed to a variety of biological, physical and chemical factors which may present potential health risks. These risks depend upon the nature of the area served by the treatment plant and the type and condition of sludge treatment process(es) in use.

This section will focus on potential health effects of exposure to microbial agents associated with the composting of municipal wastewater treatment plant sludge. Following a brief review of the literature, preliminary results will be presented of an ongoing health study of workers engaged in municipal sludge composting at several sites in the Eastern U.S.

BACKGROUND

Sewage sludge composting by the windrow method has been practiced for a number of years by the Los Angeles County Sanitation District. More recently the development of the aerated pile composting method by the U.S. Department of Agriculture, Research Station at Beltsville, Maryland, has resulted in considerable expansion of the use of sludge composting. Currently, Philadelphia, Pennsylvania; Camden, New Jersey; Washington, D.C.; Windsor, Ontario; Bangor and Portland, Maine; as well as other smaller cities are engaged in municipal sludge composting by the aerated pile method. Many other cities, including New York City, are actively considering this method of sludge treatment. A number of factors are responsible for the expansion in the use of sludge composting. Among them are the prohibition of ocean dumping of sludge after 1981, the increase in the amount of sludge being produced nationally, and the growing emphasis on the land application of wastewater and wastewater sludges.

Municipal wastewaters may contain a variety of potentially pathogenic microorganisms. Conventional wastewater treatment does not completely destroy these microorganisms, many of which are concentrated in the sludge. Therefore, sludge should be stabilized by a method such as composting, prior to its application on land. Composting is a thermophilic aerobic decomposition process. The heat generated during the composting process has been shown to effectively reduce the numbers of viable microorganisms present. Two types of composting processes are currently in use -- windrow and forced aeration pile systems (8,9). The windrow system consists of long, low piles which are aerated by periodic turning. The forced aeration pile system consists of a stationary compost pile constructed over a network

BOTTOM
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLE
AND
FIGURE
POSITIONS

BEGIN
LAST LINE
OF TEXT

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

of porous pipe attached to a blower which draws air through the pile. Temperatures in the range of 55^o-65^oC are usually attained during the composting process (8) provided the mixing or aeration is efficient. One of the most important objectives of composting is to obtain these high uniform temperatures throughout the system for sufficient duration so as to penetrate the entire mass. When this objective is fulfilled the composting process will inactivate most microorganisms including viruses.

DROPPED
HEAD:
BEGIN
SECTIONS
HERE

The heat generated during composting results in temperatures ideal for the proliferation of many thermophilic microorganisms such as actinomycetes, murcorales and in particular, Aspergillus fumigatus. Therefore, compost workers potentially may be exposed not only to the enteric pathogenic microorganisms present in raw sludge (also referred to as primary pathogens since they are capable of initiating an infection in an apparently healthy individual) but also to the thermophilic fungi and actinomycetes that proliferate during composting. Some of the thermophilic fungi are capable of infecting individuals whose defenses have been compromised (these fungi are referred to as secondary pathogens). The dust at composting sites may also contain significant quantities of lipopolysaccharide (LPS or endotoxin) derived from viable and nonviable gram-negative microorganisms which are present in sludge. In addition, the fungus Aspergillus flavus which produces aflatoxin, one of the most potent known human carcinogens, may increase in numbers during composting and the aflatoxin produced by this fungus may present an additional hazard for compost workers. Detroy et al. (10), have shown that the optimal conditions for aflatoxin production (i.e., moisture content, humidity, temperature, incubation time, aeration and nitrogen and carbohydrate content) are similar to conditions present in portions of the aerated compost pile (11). It has also been shown that aflatoxin is not destroyed by temperatures of 60-80^oC and therefore would not be detoxified by the temperatures generated during composting (10). The long term health effects of chronic exposure to the combination of microbial pathogens, microbial toxins and LPS which may be present in the work environment at composting sites have not been investigated.

Rylander et al., studied various parameters of acute and chronic inflammation in workers exposed to dust arising from heat treated sludge at a sewage treatment plant in Gothenburg, Sweden (12,13,14). These investigators observed a significant elevation in immunoglobulins (IgG, IgM, and IgA), leukocytes and platelets in workers at the sewage treatment plant as compared to a group of age matched control workers at a neighboring oil refinery. In addition, elevated levels of C-reactive protein and fibrinogen degradation products were observed in significantly greater numbers of the sewage treatment plant workers as compared to the control population. Workers at the sewage treatment plant were also reported to experience the following clinical symptoms, apparently related to heavy dust exposure: a) fever, b) purulent discharge from the eyes, c) diarrhea, and d) fatigue. The investigators postulated that the serologic changes and clinical symptoms observed in the sewage treatment plant workers may be related to endotoxin which is present in the dust arising from the heat treated sludge. They expressed concern regarding the potential health risks associated with the chronic exposure to endotoxin containing dusts in the work environment.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD,
BEGIN
SECTIONS
HERE

Rylander et al., also studied the airway immune response to inhaled endotoxin in laboratory animals (15). Rats were exposed to an aerosolized endotoxin solution (Escherichia coli 025:B6) daily for 10 days. The dose of LPS deposited in the lungs of each rat was estimated to be 0.3 μ g per day. After the 10-day exposure, elevated levels of IgG, IgM and IgA antibodies specific for E. coli 025:B6 endotoxin were demonstrated in the sera of the exposed rats. Bronchial washings from the exposed rats contained IgG and IgA antibodies directed against the E. coli 025:B6 endotoxin. In addition, the bronchial lavage fluid was also observed to contain an increased number of polymorphonuclear leukocytes (PMN) following endotoxin challenge. At cessation of exposure, the number of PMN's in the bronchial lavage fluid decreased and reached values comparable to those observed in control rats within three days. These observations suggested that inhalation of endotoxin is capable of producing an acute inflammatory response in the lungs of laboratory animals and that long term exposure to dusts containing endotoxins may lead to pathologic changes in the pulmonary parenchyma caused by the persistent inflammation. McGuire et al. (16) recently reported acute pulmonary inflammation in rhesus monkeys induced by intravenous infusion of purified bacterial LPS. These investigators have demonstrated the presence of an enzyme which cleaved components of the contact and complement systems in the lungs of the LPS challenged animals.

Rylander and his colleagues have extended their studies of the immune response to inhaled endotoxin to include workers exposed to cotton dust in cotton mills in England and Sweden (14,15). Workers with a history of exposure to cotton dust and subjective pulmonary symptoms of chest tightness were shown to have, in their nasal secretions, elevated levels of IgG and IgA antibodies specific for antigens prepared from a gram-negative bacteria isolated from cotton plants being processed in the mills. No elevation of IgG or IgA antibody levels with specificity for the antigen preparations employed were observed in nasal secretions obtained from a control population of workers with no history of exposure to cotton dust. Rylander postulated, based on his human and animal data, that chronic inhalation of endotoxin with the accompanying inflammatory response in the lungs, may play a critical role in the development of Byssinosis.

Dutkiewicz (17,18) conducted an immunologic survey of grain handlers in Poland who were exposed to high concentrations of the gram-negative bacterium Erwinia herbicola (synonym Enterobacter agglomerans) in their work environment. Workers exposed to dusts containing large numbers of E. herbicola (5×10^4 to 1×10^5 cfu/m³) had a significantly higher frequency of serum precipitating antibody directed against E. herbicola antigen preparations than unexposed individuals. Furthermore, intradermal skin testing with antigens prepared from E. herbicola revealed a higher incidence of positive skin reactions in workers exposed to E. herbicola than in unexposed individuals. Grain handlers exposed to dust containing E. herbicola and who complained of respiratory symptoms such as cough, dyspnea, and wheezing, were shown to have a higher incidence of positive skin and precipitin reactions to E. herbicola antigens than asymptomatic exposed workers. This study provides further evidence to suggest that exposure to gram-negative LPS by inhalation is capable of inciting a systemic immune

BEGIN
LAST LINE
OF TEXT

3/8"

16

BOTTOM OF
IMAGE AREA
DIMENSIONS
FOR TABLES
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

response and that it may play a primary role in the development of chronic pulmonary disease in exposed individuals.

DROPPED
HEAD;
BEGIN
SECTIONS
HERE

Lundholm and Rylander (19) recently reported on a study of 11 workers at a plant where municipal sewage sludge and household garbage were crushed, milled, and allowed to compost for six months. Gram-negative bacteria were reported to be present in large numbers where garbage was loaded onto the conveyor belts (28,000 cfu/m³) and even higher near the mill outlet (25,000-500,000 cfu/m³). Elsewhere at the plant they ranged from an average of 330 to 11,000 cfu/m³. At the central water treatment plant levels averaged 20 cfu/m³. Six of the 11 compost plant workers reported symptoms of nausea, headache, fever or diarrhea compared to only two of the 41 water treatment plant employees who served as controls.

Environmental Monitoring

Millner, Bassett and Marsh (20) recently reported on their monitoring of airborne spores of A. fumigatus at two compost sites at Beltsville, Maryland and at the Blue Plains Wastewater Treatment Plant in Washington, D.C. By volumetric sampling they were able to determine that the dispersal of the spores from composting sewage sludge and wood chip mixtures behaved as a Gaussian plume. A major source of airborne spores was found to be the front-end loader which was estimated to aerosolize 4.6×10^6 A. fumigatus spores/second when moving and dropping compost. The sampling instrument used was the six-stage Andersen sampler with the sampling orifice directed into the wind and with the top cone removed to collect more nonrespirable particles (21). Other investigators (22) including us, (23) have neither removed the top cone nor tilted the sampler in their sampling procedure. At the Beltsville compost site airborne concentrations of A. fumigatus between three and 30 meters downwind of a compost pile being agitated by a front-end loader were reported to range from 1400 to 3100 cfu/m³. Fifteen minutes after agitation, levels were not above 39 cfu/m³.

Potential Health Effects of Aspergillus fumigatus

Aspergillus species are ubiquitous in the environment of most countries of the world. The fungus grows well on a variety of substrates, including stored hay or grain, decaying vegetation, soil, and dung. A. fumigatus grows well at 45°C or even higher, making it one of the most common microorganisms found in compost sites (3). A. fumigatus, and others of the A. species, have been shown to be capable of causing disease in both normal and compromised individuals. Therefore, workers involved in the composting process may be exposed to potential health risks due to their exposure to A. fumigatus.

The term "Aspergillosis" has been used to describe illness attributed to antigenic stimulation, colonization, or tissue invasion by A. species. Aspergillosis is usually acquired by inhalation of airborne spores. These spores (conidia) are small enough (2.5-3.0µm for A. fumigatus) to reach alveoli or to gain entrance to paranasal sinuses. The disease varies in severity from an incidental, saprophytic relationship with the host to a fulminating, fatal infection.

BEGIN
LAST LINE
OF TEXT

BOTTOM
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD;
BEGIN
SECTIONS
HERE

The dose (number of cfu), portal of entry, and the immune status of the host are thought to be major determinants in the course of the infection caused by A. species. Immunosuppressed patients are at a greater risk to infection by A. fumigatus (24). Serious and often fatal invasive infections caused by A. fumigatus have been reported in immunosuppressed patients following kidney transplanation and in patients with leukemia or lymphoma receiving chemotherapy. Individuals receiving antibodies or adrenal cortical hormones have also been shown to have a higher incidence of mycotic infections. Additional predisposing factors to aspergillosis appear to be the presence of malignant or other debilitating disease, leukopenia or granulocytopenia, other infections, pneumonitis and underlying pulmonary disease (24).

Exposure of atopic individuals, who have a history of asthma, to spores of A. fumigatus may result in a disease known as allergic bronchopulmonary asperillogis. Colonization of the bronchi in these patients with Aspergillus results in episodic bronchial plugging which appears to lead to areas of sacular bronchiectasis. These patients may produce sputum plugs which often reveal fungal mycelia on microscopic examination. Laboratory abnormalities in these patients include significant eosinophilia of blood and sputum, marked elevation of total serum IgE, and serum precipitating antibody to Aspergillus antigens. These patients usually have an immediate type skin response to Aspergillus antigen. Persistent bronchopulmonary aspergillosis may result in irreversible complications such as bronchiectasis and pulmonary fibrosis.

The pathophysiology of allergic bronchopulmonary aspergillosis is thought to be mediated by a combination of both Type I and Type II immunologic reactions. IgE-sensitized mast cells in the bronchi react to antigens from Aspergillus colonies growing in the bronchi. These mast cells release histamine, slow-reacting substance of anaphylasix and eosinophilic chemotactic factor leading to bronchospasm, increased permeability of the bronchial mucosa, absorption of Aspergillus antigen into the circulation, and pulmonary and peripheral blood eosinophilia. The absorbed Aspergillus antigen can react with IgG, resulting in the formation of antigen-antibody complexes and complement fixation. This in turn, may lead to chronic inflammation in the bronchi and peribronchial tissues with eventual bronchial destruction, bronchiectasis, and pulmonary fibrosis.

Due to the wide distribution of A. species in the environment, exposure to Aspergillus must be nearly universal, but disease, either invasive or allergic, is uncommon. As indicated above, a complex interaction between host factors and the challenge dose of A. species spores appears to determine the course of events which follow exposure to Aspergillus.

To date, no known adverse health effects have been detected among compost workers due to exposure to thermophilic fungi and/or actinomycetes. The Los Angeles County Sanitation District has been composting sewage sludge for many years using the windrow method. The compost produced is marketed by Kellogg Supply Co., Inc., for use in a wide variety of applications. In the several decades of the operation of this system,

BEGIN
LAST LINE
OF TEXT

BUTTON ON
IMAGE AREA
OUTSIDE
DIMENSIONS
FOR TABLE
AND FIGURE
CAPTIONS

there have been no reports of adverse health impacts (9), however, due to the apparent transient nature of the work force the efficiency of the reporting system is probably low.

Composting by the forced aeration method has been carried out in Beltsville since March 1973, and for shorter periods of time in other locations. In the accumulated time of system operation and worker and user exposure, there have thus far been no reported cases of disease resulting from exposure to compost.

ELEMENTS OF COMPOST WORKERS STUDY

This investigation was designed to evaluate the health effects related to exposure to nonviable substances and viable microorganisms present in dust arising from the composting of sewage sludge. The clinical and serologic evaluation of workers exposed to dust at the composting site included: 1) comprehensive history and physical examination; 2) health questionnaires; 3) illness monitoring; 4) liver and kidney function profiles; 5) anterior nares and oropharyngeal swab cultures; 6) chest X-ray; 7) complete blood count with differential; 8) determination of antibodies directed against *A. fumigatus*; 9) determination of antibodies directed against lipopolysaccharide (LPS) present in compost samples; 10) skin testing with *A. fumigatus* antigen; 11) pulmonary function tests; 12) quantitation of total IgG, IgM, IgA, and IgE; and 13) determination of C-reactive protein and levels of C3 and CH₅₀. Health questionnaires, anterior nares and oropharyngeal swab cultures and sera for each of the serologic tests were obtained five times during 1979, the first year of the investigation. Environmental monitoring for viable particles was also conducted several times during the first year. The scheduling of the above elements of the study protocol is outlined in Table 1.

Population Groups Selected

Workers at sludge composting facilities in Camden, N.J., Beltsville, M.D. and Washington, D.C. were selected because of their geographic closeness and the willingness of their organizations to cooperate with the study. Control workers were recruited either from sewage treatment plants producing the sludge, if the job locations were judged to be sufficiently distant from the compost operations, or from other nearby sewage treatment plants. Compost treatment facilities in Camden, N.J. and Washington, D.C. are operated by the Camden County Municipal Utilities Authority and by the District of Columbia Department of Environmental Services, respectively. In Camden the compost unit is located at the main wastewater treatment plant and in Washington, D.C. at the Blue Plains Wastewater Treatment Plant. Permission was not received from the U.S. Department of Agriculture to recruit their compost-exposed employees at the Beltsville Agricultural Research Center, but it was received from the Maryland Environmental Services which employs many of the workers involved with the Beltsville compost-related activities. Nonexposed workers in the Camden area were recruited from a small secondary wastewater treatment plant in Camden, the Baldwin Run Plant. In the Washington, D.C.-Beltsville, MD

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
PAGE AREA

TABLE 1. SCHEDULING OF ELEMENTS OF COMPOST WORKERS STUDY

Study element	1	5	Month		
			7	9	12
Comprehensive history	X				
Physical examination				X	
Illness monitoring	X	X	X	X	X
Anterior nares and oropharyngeal swabs for <u>A. fumigatus</u>	X	X	X	X	X
Chest X-ray			(ONCE)		
Complete blood count with differential	X	X	X	X	X
Fungal serology (<u>A. fumigatus</u> , <u>A. carneus</u> , <u>A. niger</u> and <u>A. flavus</u>)*	X	X	X	X	X
Determination of antibody directed against Lipopolysaccharide (LPS) prepared from compost	X	X	X	X	X
Skin testing with <u>A. fumigatus</u> antigen preparation**				X	
Pulmonary function testing	X	X	X	X	X
Immunochemical determination of CRP, C3, and immunoglobulins (IgM, IgG, and IgA)	X	X	X	X	X
IgE determination					X
Environmental monitoring		X	X	X	X
Renal and liver profiles	X				X

*Aspergillin, Meridian Diagnostics, Cincinnati, Ohio.

**A. fumigatus allergic extract for scratch test (1:10 W:V)
Hollister-Stier Lab., Spokane, Washington.

BEGIN
LAST LINE
OF TEXT

RETURN TO
ORIGIN AREA
OUTSIDE
CORNER OF
PAGE FOR
REVISIONS

located about 10 miles down the Potomac River from the Blue Plains Plant. The Beltsville compost facility treats a small portion of the sludge produced at the Blue Plains Plant. Another portion of the Blue Plains Plant sludge is trenched elsewhere in Maryland. The Camden compost facility treats sludge from the treatment plant at which it is located as well as that from the smaller Baldwin Run Plant. The size and type of treatment facilities from which these study participants were recruited are given in Table 2. A total of 170 workers were recruited from these plants for the study that is now being reported. About 100 additional low exposure workers have been recruited from the Blue Plains Plant.

TABLE 2. SIZE AND TYPE OF TREATMENT FACILITIES FROM WHICH PARTICIPANTS WERE RECRUITED

Location	Wastewater		Compost		Date Started
	Size (MGD)	Type	Size (dry tons/day)		
Camden, NJ					
Main Plant	30	Primary	15		5/78
Baldwin Run	4	Secondary	-----None-----		
Beltsville, MD	-----None-----		15		3/73
Washington, D.C. (Blue Plains)	300	Secondary	75		2/79
Piscataway, MD	30	Secondary	-----None-----		

Compost workers and control subjects in Philadelphia, PA (30) and Bangor and Portland, ME (20), have also been recruited. Results from these workers will be included in a future report.

METHODS

Clinical and Serological

The study was designed to evaluate the participants for objective and subjective symptoms and signs possibly related to exposure to dust associated with the composting operation. Participants received physical examinations once during the first year of the study. In addition, a detailed medical and occupational history, which included reference to contact with sewage sludge composting operations, or any other occupational dust and/or waste exposure was obtained from each participant. This data base allowed ranking of the participants in this investigation in terms of length and severity of exposure to the environmental dust and other factors associated with composting. A health questionnaire, specifically designed to determine the occurrence of hypersensitivity, respiratory, gastrointestinal and mucocutaneous disorders associated with dust exposure, was obtained from

the participants during the first, fifth, seventh, nine and twelfth months of 1979. LPS (endotoxin) was extracted from bulk compost samples from each composting site by the method of Westphal (25). The serum from the study population was tested for the presence of antibodies directed against each of the LPS preparations by the enzyme-linked immunosorbent assay (ELISA). Because of the difficulty in demonstrating infection with members of Aspergillus species, four parameters were employed to detect infection with these fungi: 1) determination of precipitating antibody directed against A. fumigatus, A. carneus, A. niger and A. flavus in the serum of the study population, 2) determination of skin test reactivity to an extract of A. fumigatus, 3) semi-quantitative sputum cultures for A. fumigatus, and 4) evidence of transient or migratory infiltrates on chest X-ray.

Precipitating antibody to A. fumigatus, A. flavus, A. carneus and A. niger (Aspergillin, Meridian Diagnostics, Cincinnati, Ohio) in the serum of the study population was measured semi-quantitatively by titration of antibody using the counter immunoelectrophoretic technique (26). Antigens of A. fumigatus used in the determination of specific IgG antibody by the ELISA were prepared by the method of Coleman and Kaufman (28) and further purified by gel filtration on Sephadex G-200. The first peak obtained by gel filtration of the antigen preparations were be used for determination of precipitins in the serum samples tested. Sera positive for A. fumigatus were kindly provided by Dr. J. W. Rippon, University of Chicago Hospital, Chicago, IL, and were used as positive controls for precipitin determinations. Serum samples for fungal precipitin determination were obtained from the study population during the first, fifth, seventh, ninth and twelfth months of the first year of the investigation.

Skin reactivity to Aspergillus antigen was determined by scratch test, using Aspergillus antigen (Hollisten-Stein Corp., Spokane, WA) in 1:10 weight/volume concentration. The skin test was performed during the twelfth month of the study. Determination of total IgE titers were performed by the ELISA method using sera collected during December 1979.

Anterior nares and oropharyngeal swabs were obtained from the study population during the fifth, seventh, ninth, and twelfth months of the first year of the investigation and cultured for A. fumigatus. Sputum specimens were obtained during the first month but were discontinued because of the difficulty in obtaining adequate samples. Due to the widespread distribution of A. fumigatus, semi-quantitative determination of A. fumigatus in the samples obtained from the study and control groups was performed. Quantitation of the number of A. fumigatus in the samples may provide an index of the degree of colonization between the groups participating in the study.

Transient or migratory pulmonary infiltrates are frequently observed in individuals with hypersensitive lung diseases. A chest X-ray was obtained on participants once during the first twelve-month period and any infiltrative process or other lung pathology was recorded. In the future, prior chest X-rays of the study participants will be reviewed, if possible, and any positive findings will be correlated

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
MARGIN
AREA

with the clinical diagnosis made at the time the X-ray was taken and with periods of exposure to the composting operation.

CHOPPED
HEAD-
BEGIN
SECTION
HERE

Chronic exposure to microbial antigens present in the dust arising from the composting of sewage sludge may induce a local and/or systemic inflammatory response as well as an elevation of one or more classes of circulating immunoglobulins. C-reactive protein and C3, serum proteins which are often elevated during inflammatory processes, were quantitated by radial immunodiffusion (28). Hemolytic complement was also assayed (26) since it may be reduced in immunological phenomena involving antigen-antibody complexes. The concentration of total IgG, IgM, and IgA was quantitatively determined in the sera of the study population by radial immunodiffusion (28).

The study population was also evaluated for evidence of hypersensitive pulmonary disease or other lung diseases which may be associated with prolonged exposure to the dust arising from the composting operation. Each participant in the proposed investigation underwent pulmonary function testing. When possible, pre- and post-shift testing was performed. It was felt that pulmonary function testing in this manner would detect bronchial sensitivity associated with exposure to dusts in the work environment.

Environmental Monitoring

Viable particle collection from air samples was performed using the six-stage Andersen cascade impactor designed for that purpose. Air was drawn through the samplers by 12 volt D.C. pumps, which were powered by 12 volt motorcycle batteries. The pumps, attached to an assembled sampler loaded with petri dishes containing agar, were calibrated against a dry gas meter to pull one cubic foot of air per minute (CFM), as recommended by the manufacturer. The samplers were mounted on tripods such that samples were collected at a height of five feet, which approximate the breathing zone of the average worker. Sampling times were adjusted in an attempt to obtain colony numbers which were optimal for counting. The volume of air sampled was calculated from the sampling time and the flow rate of the pumps. Total colony counts for bacterial and fungal plates were obtained by direct colony count, unless excessive colony numbers precluded this procedure. If colony numbers precluded counting all colonies, the colonies on 1/4 or 1/2 the plate were counted and the count was multiplied by the appropriate number to obtain a total plate count.

Bacterial samples were collected on Trypticase soy agar (TSA) manufactured by Baltimore Biological Laboratories (BBL), Cockeysville, MD. The medium was prepared according to the manufacturers instructions, autoclaved at 121°C for 15 minutes, cooled to 45-50°C and dispensed into petri dishes. Prior to use, TSA plates were incubated at 36°C for 24-48 hours to detect contamination and minimize surface moisture due to condensation.

Fungal samples were collected on modified Czapek-Dox medium. The medium was prepared by adding 35 grams of Czapek-Dox broth (Difco Laboratories, Detroit, MI), 20 grams agar (Difco), and 15 grams Oxgall (Matheson, Coleman and Bell, Cincinnati, OH) to 1000 milliliters distilled water, adjusting the

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
PAGE AREA
OPTIONAL
DIMENSIONS
FOR TABLES
AND ILLUSTRATIONS

pH to 7.3 and autoclaving at 121°C for 15 minutes. Prior to dispensing, the medium was cooled to 45-50°C and 50 µg/ml Streptomycin, 50 µg/ml Chloramphenicol and 20 units/ml Penicillin were added to retard bacterial growth. These plates were incubated at room temperature or occasionally at 36°C to minimize moisture.

Aerosol samples obtained on TSA plates were shipped directly (within 24 hours) to the Research Surgical Bacteriology Laboratory at the University of Cincinnati, where they were incubated at 36°C for 24 hours. At the end of the incubation period, total bacterial counts were made from the TSA plates which were then replicate plated onto three different types of media: KF streptococcal agar (BBL), M - FC agar (BBL), and M-Endo agar (LES) (BBL) (30).

KF streptococcal agar is a selective medium which supports the growth of fecal streptococci (Lancefield's serological groups D and Q) while inhibiting the growth of most gram-negative and other gram-positive bacteria. The presence of pink/red colonies after 48 hours at 36°C is indicative of fecal streptococci. All growth on KF streptococcal agar was re-replicated to Bile-Esculin agar (Difco) and tested for catalase activity. Gram-positive cocci which grew as brown/black colonies on Bile-Esculin agar, and which failed to decompose a three percent solution of hydrogen peroxide, were considered to be confirmed fecal streptococci.

The purpose of replication onto M-FC agar was to determine the presence or absence of fecal coliforms. These plates were incubated at 44.5°C for 24 hours and the blue to blue green colonies were transferred to a Lauryl sulfate broth (BBL) which was incubated 24-48 hours at 37°C and to EC broth (BBL) which is incubated for 24 hours at 44.5°C. Growth with gas in these media confirmed the presence of fecal coliforms.

In order to determine total coliform counts, the TSA plates were replicated onto M-Endo agar (BBL) and incubated at 37°C for 24 hours. The colonies with a green sheen were picked to Lauryl sulfate broth and Brilliant green bile broth (BBL). These media were incubated at 37°C for 48 hours, $\pm$ 3 hours. Growth with gas confirmed the presence of coliforms.

Fungal plates, upon reaching the laboratory, were incubated at 42.5 to 44.5°C for 48 hours and total plate counts were performed. The microorganisms were separated into *A. fumigatus* and "other thermophilic microorganisms" on the basis of microscopic examination and colony color and morphology.

RESULTS

In This report only a preliminary analysis of a portion of the data from participants at Camden, Beltsville, Washington, D.C. (Blue Plains) and Piscataway is available. Data from workers in Philadelphia, PA, Bangor and Portland, ME and from the additional lower exposed Washington, D.C. volunteers will be presented in a future report.

Populations Recruited

A summary of the 173 workers recruited from Camden, N.J., Beltsville, MD,

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
PAGE
MARK

CONSIDER
THIS AS
A GUIDE
ONLY

Washington, D.C. (Blue Plains) and Piscataway, MD is presented in Tables 3, 4, 5 and 6, respectively. Initial recruitment began in late January, 1979 in Camden, N.J. and in March for Beltsville, MD, Washington, D.C. and Piscataway, MD. The participants from each of these areas have been assigned compost exposure categories: high, intermediate, or control. Assignment to a particular category was based on interviews and direct observations. Workers who are directly involved in compost operations as their major activity were assigned to the high exposure category. The intermediate category applies to workers either routinely working within about 100 meters of a compost operation or who are sometimes involved with composting operations but not as the major part of their work. These categories are relative ones and are area specific; that is an "intermediate exposure" workers at the Camden compost plant would not necessarily have the same exposure as an "intermediate exposure" worker at Blue Plains.

In Camden the intermediate exposure category was used for workers at the Main Plant who were not involved in the compost operation. Control workers in Camden were from the Baldwin Run Plant. There were 21 Camden participants in the high exposure category, 22 in the intermediate and 14 in the low exposure group. At Beltsville, MD there were 10 people in the high exposure group working at the compost site and there were eight in the intermediate group who primarily worked with compost samples at a research laboratory some distance from the compost and on occasion visited the compost site for sample collection and other purposes. Included in the high exposure group was one person whose job was based in a trailer located at the compost site. In Washington, D.C. 14 compost workers have been recruited in the high exposure group. An intermediate exposure group consisted of 27 workers whose jobs occasionally required them to repair equipment in the Blue Plains compost pit and on occasion at the Beltsville compost site. A control group of 11 workers was recruited who report to a pumping station several miles from the Blue Plains Plant but spend a portion of their time at the Blue Plains Plant maintaining the pumps in the raw sewage inlet station and in the air blower building, both of which were several hundred meters from the compost pit. The 40 participants recruited at the Piscataway, MD plant were engaged in various operational and laboratory functions. All have been assigned to the control category since no composting operations are involved at their plant. The median age for the Camden groups ranged from 28 to 37 years, at Beltsville, from 27 to 47, at Blue Plains from 31 to 42, and at Piscataway the median age of the one group was 30 years. The gradations of exposure at Camden, Beltsville and Blue Plains allowed for comparison of results between workers at these locations. In addition, Blue Plains workers were compared to the control group at nearby Piscataway, MD.

Air Sampling

During the period May-December, 1979 viable particle air sampling was conducted at several locations at each of the treatment facilities involved in the study. Results are summarized for *A. fumigatus* and "Other Thermophilic Microorganisms" in Table 7, for Fecal Streptococci and Fecal Coliforms in Table 8, and for Total Coliform and Total Bacteria in Table 9. At the Blue

BEGIN
LAST LINE
OF TEXT

3/8

BOTTOM
MARGIN AREA
OUTSIDE
DIMENSION
FOR TABLES
AND FIGURE
CAPTIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
PAGE
AREA

TABLE 3. CAMDEN WORKERS: (AGE, RACE, SEX AND EXPOSURE)

Exp.	Whites			Non-whites			Total		
	No.	Med.	Range	No.	Med.	Range	No.	Med.	Range
High	19(1)	28	19-61	2	55	48-61	21(1)	29	19-61
Int.	19	26	19-70	9	48	25-68	28	28	19-70
Cont.	7	27	23-61	7	43	22-64	14	37	22-64

Number of which are females appear in parentheses.

TABLE 4. BELTSVILLE WORKERS: (AGE, RACE, SEX AND EXPOSURE)

Exp.	Whites			Non-whites			Total		
	No.	Med.	Range	No.	Med.	Range	No.	Med.	Range
High	9(1)	50	29-59	1	43		10(1)	47	29-59
Int.	8(2)	27	25-65	0			8(2)	27	25-65
Cont.	0			0			0		

Number of which are females appear in parentheses.

TABLE 5. BLUE PLAINS WORKERS: (AGE, RACE, SEX AND EXPOSURE)

Exp.	Whites			Non-whites			Total		
	No.	Med.	Range	No.	Med.	Range	No.	Med.	Range
High	1	32		13(1)	37	20-55	14(1)	37	20-55
Int.	5	35	26-45	22(6)	31	23-49	27(6)	31	23-49
Cont.	2	36	29-42	9	42	23-51	11	42	23-51

Number of which are females appear in parentheses.

TABLE 6. PISCATAWAY WORKERS: (AGE, RACE, SEX AND EXPOSURE)

Exp.	Whites			Non-whites			Total		
	No.	Med.	Range	No.	Med.	Range	No.	Med.	Range
High	0			0			0		
Int.	0			0			0		
Cont.	18(1)	28	22-63	22(6)	31	25-47	40(7)	30	22-63

Number of which are females appear in parentheses.

Plains and Main Camden plants results were summarized for samples collected in the vicinity of the compost area and at locations more distant from the compost areas. Because of the much larger size of the Blue Plains Plant, "Other Areas" for it were at least 150 meters from the compost area while they were only at least 50 meters away at the Camden plant. (At the Camden plant, all areas of the plant were within 150 meters from the compost area.) Samples were collected at each plant on from three to six days except for Beltsville where samples were collected on only one day. Only colonies from plates 3-6 (respirable size range) from the Andersen samplers were included in Tables

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
PAGE AREA
FOR TABLES
AND FIGURES

TABLE 7. SUMMARY OF 1979 AIR MONITORING FOR RESPIRABLE CONCENTRATIONS OF A. FUMIGATUS AND OTHER THERMOPHILIC MICROORGANISMS (COLONY FORMING UNITS PER CUBIC METER, CFU/M³)

Area	No. samples	A. fumigatus		Concentrations Other thermophilic microorganisms	
		Range	Average	Range	Average
Metro. Washington, D.C.					
Beltsville, Compost	4	48-131	81	0-34	17
Blue Plains, Compost	25	0-475	52	0-12,000	1060
Blue Plains, Other Areas*	14	0-21	3	0-246	73
Piscataway Plant	15	0-18	3	0-5	<1
Camden, N.J.					
Main Plant, Compost	13	0-2940	918	0-580	146
Main Plant, Other Areas**	6	0-126	24	0-7	2
Baldwin Run Plant	11	0-52	14	0-7	1

*At least 150 meters from compost area.

**At least 50 meters from compost area.

TABLE 8. SUMMARY OF 1979 AIR MONITORING FOR RESPIRABLE CONCENTRATIONS OF FECAL STREPTOCOCCI AND FECAL COLIFORMS (COLONY FORMING UNITS PER CUBIC METER, CFU/M³)

Area	No. samples	Concentrations		
		Fecal streptococci		Fecal coliforms
		Range	Average	Range Average
Metro. Washington, D.C.				
Beltsville, Compost	4	0-23	6	0
Blue Plains, Compost	4	11-79	48	0
Blue Plains, Other Areas*	0			
Piscataway	6	0-6	1	0-3 1
Camden, N.J.				
Main Plant, Compost	8	0-482	85	0
Main Plant, Other Areas**	2	0-5	3	0-5 3
Baldwin Run Plant	6	0-25	8	0-21 5

*At least 150 meters from compost area.

**At least 50 meters from compost area.

TABLE 9. SUMMARY OF 1979 AIR MONITORING FOR RESPIRABLE CONCENTRATIONS OF COLIFORMS AND TOTAL BACTERIA (COLONY FORMING UNITS PER CUBIC METER, CFU/M³)

Area	No. samples	Concentrations		
		Total coliforms		Total bacteria
		Range	Average	Range
Metro. Washington, D.C.				
Beltsville, Compost	4	0	0	5,550-16,800
Blue Plains, Compost	4	0	0	8,070-18,100
Blue Plains, Other Areas*	0			
Piscataway, Plant	6	0-27	12	564-1330
				815
Camden, N.J.				
Main Plant, Compost	8	0-56	8	590-5170
Main Plant, Other Areas**	2	0-10	5	443-524
Baldwin Run Plant	6	0-35	10	196-3380
				2720
				484
				1460

*At least 150 meters from compost area.

**At least 50 meters from compost area.

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

7-9. Concentrations of A. fumigatus varied considerably and on average were higher in compost areas than in noncompost areas. Concentrations at areas at least 150 meters from the compost pit at the Blue Plains Plant were about the same as at the Piscataway Plant where no composting occurs. At the Main Camden Plant, A. fumigatus levels in areas at least 50 meters from the compost pit were on average much lower than concentrations in the compost area, but were on occasion higher than those at the Baldwin Run Plant. Concentrations of A. fumigatus at Camden were generally higher than those at Beltsville and Blue Plains. These differences may be due in part to differences in compost activities underway at the time of the sampling or to differences in precipitation levels prior to sampling. Two days prior to one of the Blue Plains sampling days, about four inches of rainfall occurred in the vicinity of the plant as tropical storm David passed through the D.C. area. About six percent of all colonies of A. fumigatus were found on plate 6 and were thus thought to be small enough to reach the alveoli. This compares to less than one percent found to be in that size range by Millner et al. (20). About 80 percent were found to be in the respirable size range (plates 3-6) which is practically the same as that reported by Millner et al. (20), 79 percent.

DROPPED
HEAD,
BEGIN
SECTION
HERE

In Camden the two treatment plants are clearly distinguishable from each other with respect to A. fumigatus levels. At the main Camden plant A. fumigatus levels were considerably higher at compost areas than elsewhere in the plant where samples were collected. Similarly, A. fumigatus concentrations at the Piscataway Plant and at areas of the Blue Plains Plant remote from the compost pit were clearly lower than levels reached at the Beltsville and Blue Plains composting sites.

Concentrations of other thermophilic microorganisms varied widely but were generally higher at the compost sites.

Fecal streptococci were detected at each of the areas where they were measured and were generally higher at the compost areas (Table 8). Fecal coliforms were infrequently detected (Table 8). Coliforms (Table 9) were not detected in samples collected at Beltsville and Blue Plains. Concentrations were about the same at Piscataway as they were at the Camden sampling locations (averages ranged from 5 to 12 cfu/M³). Concentrations of total bacteria ranged somewhat higher at compost sites than at other sites but were above 10² cfu/M³ in all samples.

A. fumigatus in Study Participants

Culturing for of A. fumigatus colonies was performed on sputum specimens or from swab cultures of the anterior nares and oropharynx obtained from study participants. Since not all participants were available for testing during each of the five visits, between one and five determinations were made for each individual. A summary of the highest value for each individual, according to location and exposure category, is presented in Table 10. The number of colonies observed are expressed as either zero or greater than or equal to one. Statistical analyses by the chi-square test, or Fisher's exact test (31) when appropriate, were performed between results for the various exposure groups and combinations of exposure groups in each city and between groups for

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA,
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

3/8"

30

EPA Form 2350-4 (4-80)

(PREVIOUSLY CEN. EPA FORM 337)

PAGE NUMBER

U.S. GOVERNMENT PRINTING OFFICE: 1980-384-639

TYPING GUIDE SHEET

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTIONS
HERE

TABLE 10. DISTRIBUTIONS AND ANALYSES OF STUDY PARTICIPANTS ACCORDING TO THE HIGHEST VALUE OF ASPERGILLUS FUMIGATUS (AF) COLONIES OBSERVED ON CULTURES TAKEN DURING 1979

	Exposure Categories			Exposures Compared	Testing of Hypothesis of Independence	
	1	2	3		χ^2 Test	Fisher's Exact Test
Camden						
AF ≥ 1	16	5	1	1:2	$<.0005$	
AF = 0	5	20	12	1:3	$<.0005$	
				1:2,3	$<.0005$	
				2:3		$\sim .314$
Blue Plains				1:2	$<.05$	
AF ≥ 1	8	7	0	1:3		$\sim .002$
AF = 0	5	20	11	1:2,3	$<.005^*$	
				2:3		$\sim .070$
Blue Plains & Piscataway				1:2	$<.05$	
AF ≥ 1	8	7	2	1:3		$<.0001$
AF = 0	5	20	48	1:2,3	$<.005^*$	
				2:3		$\sim .007$
Beltsville				1:2		$\sim .004$
AF ≥ 1	7	0				
AF = 0	3	8				
All Sites Combined				1:2	$<.0005$	
AF ≥ 1	31	12	3	1:3	$<.0005$	
AF = 0	13	48	60	1:2,3	$<.0005$	
				2:3	$<.01$	

*Small cell expected value renders the validity of the χ^2 test "borderline."

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

all areas combined. The null hypothesis tested is that the probability of a positive count is independent of exposure category. Statistically significant differences were found between many of the groups with those in exposure category one having more positive determinations than either those in category two or category three. Workers in category 2 had more positive values than those in group three and the results were statistically different for Blue Plains and Piscataway combined and for all sites combined.

DROPPED
HEAD
BEGIN
SECTION
HERE

The differences among the groups in A. fumigatus colonies verified the overall validity of the exposure categories. Even though viable A. fumigatus (AF) spores are ubiquitous in the environment, the increase, above ambient levels in their concentration at or near compost sites was apparently large enough to result in a higher degree of incidence among workers with compost exposure than in those without. Combining all sites, the percentages of study participants exhibiting at least one positive value for AF colonies, distributed by exposure category, were category one (high exposure) 70%; category two (intermediate exposure) 20%; category three (control) 5%.

Liver Function and Total Immunoglobulin Determination

Preliminary analyses of the highest results obtained for each participant on tests for the enzymes serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), measures of liver function, and levels of total IgG, IgE and IgM, did not reveal any differences among exposure groups at any of the sites. Combining data by exposure group for all sites again did not reveal any significant difference among exposure groups. A more detailed examination of the data may reveal differences in mean values for the various exposure groups, however.

Delayed Hypersensitivity to Aspergillus Antigens

Skin reactivity to Aspergillus antigen was determined on 109 participants in December, 1979. Only five persons tested positive, three compost workers, one intermediate-exposed worker and one in a control group. These numbers were too small for statistical analyses.

C-Reactive Protein (CRP)

Preliminary analysis of the highest CRP value for each individual was performed on two groupings of the data: (1) <1 mg% and ≥ 1 mg% and (2) positive and nonreactive. For the first grouping there were no differences among exposure groups at the various sites or for all sites combined. Comparisons of the positive and nonreactive results revealed only one borderline statistically significant result ($p = .053$) by the Fisher's exact test: Four of nine compost workers at Beltsville had positive values for CRP compared to none of eight intermediate-exposed workers at Beltsville.

Eosinophil Count

Preliminary analyses of the highest absolute eosinophil count for each of the participants did not reveal any differences among the exposure groups at the various sites, separately or combined.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA -
OUTSIDE
DIMENSION
FOR TABLE
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP LINE
SPACE
HERE

Chest X-Ray Findings

X-rays were obtained from 74 participants and were reviewed by a radiologist at the University of Cincinnati. Although a total of 17 abnormal findings were observed, only two were suggestive of occupational exposure or smoking, one a compost worker and one a control.

UNWRAPPED
HEAD
FROM
SECTIONS
HERE

Antibody to *A. fumigatus*

Specific IgG directed towards *A. fumigatus*, as determined by the ELISA, was compared by exposure group for each site separately. Statistical analyses were performed by the Mann-Whitney one-tailed ranking test. At no site did the compost workers exhibit statistically higher values.

Antibody to Lipopolysaccharide (LPS)

LPS was prepared from compost samples obtained from the Camden site, and from the Beltsville-Blue Plains sites combined, by the Westphal method and tested against workers sera to determine possible responses to LPS present in the compost material. Specific IgG directed against the LPS was determined by the ELISA. Statistical analysis was by the Mann-Whitney one-tailed ranking test. Antibody titers in the Camden compost workers (group one) directed against LPS prepared from compost obtained from Camden were not higher than in the control workers (group three). However, in Beltsville, the compost workers had higher antibody titers against LPS prepared from compost obtained from the Blue Plains-Beltsville compost sites, than the intermediate group ($p = .04$). At Blue Plains, antibody titers in the compost workers were higher than in the intermediate group ($p = .025$); when group three (control) workers at Piscataway and Blue Plains were included the significance was still $p = .02$.

Health Effects Associated with Composting

A review of the medical histories, physical examinations and chest X-rays of compost workers participating in the study has thus far revealed one worker with several health problems which may be associated with exposure to microbial pathogens and dust present at the composting site. Approximately one year after starting employment at a compost site, the worker developed chronic otitis media of the right ear, with sclerosis of the right mastoid and erosion of the posterior wall of the right external canal demonstrated on X-rays of the mastoid. Over a period of several months the infection gradually progressed, with erosion of 70% of the right tympanic membrane, despite topical antibiotics, local debridement, and a right tympanomastoidectomy. Aspergillus niger, a fungus which has been reported present in compost, was the only pathogen isolated from the purulent discharge present in the right ear. This worker also has small irregular shadows in both lungs suggestive of parenchymal disease. Since this individual states that he has never smoked, the pulmonary abnormalities observed on the chest X-ray may be job related. Except for evidence of the right chronic otitis, the physical examination of this worker was normal, as were his pulmonary function studies on three

BEGIN
LAST LINE
OF TEXT

BOTTOM LINE
SPACE
HERE
FOR TABLES
AND FIGURES

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
HERE

different occasions. The worker also complained of symptoms suggestive of chronic sinusitis, however, the relation of the onset of these symptoms and his exposure to composting could not be definitely determined.

DISCUSSION AND CONCLUSION

GROUPED
HEAD-
ING
SECTION
HERE

Workers at sludge compost sites are exposed to a variety of fungal and bacterial pathogens, as well as toxins of microbial origin, in their work environment. No information is currently available regarding the potential long-term effects of this exposure on the health of these workers. This report has reviewed current literature related to host responses to microorganisms and microbial toxins known to be present at compost sites and has presented preliminary results of a study designed to evaluate potential health effects of exposure to microbial factors present at compost sites.

Results of the environmental monitoring demonstrated that workers at each of the compost sites studies were exposed to markedly elevated numbers of *A. fumigatus* spores. This observation was supported by the high frequency of isolation of *A. fumigatus* from oropharyngeal and anterior nares cultures obtained from the compost workers when compared to the other two exposure groups. However, the presence of *A. fumigatus* in the anterior nares and oropharyngeal cultures did not correlate with antibody response directed against *A. fumigatus* in those workers with positive cultures or among the three exposure groups. One worker in the study, employed at a compost site, was found to have a local infection involving his right ear caused by *A. niger* and chest X-ray changes compatible with an occupationally-related disorder. It has not been possible to document whether the ear infection or the chest X-ray findings were directly related to exposure to microbiologic agents present at the compost site.

Due to the large numbers of gram-negative bacteria in the sewage sludge which is composted, it was assumed that workers at compost sites would be exposed to relatively large amounts of LPS (endotoxin) in the dust at the compost site. The only means by which this exposure could be documented was by the viable counts of gram-negative microorganism (Fecal Coliforms) collected during environmental monitoring. These levels may be artificially low since a major component of the LPS in dust may be comprised of nonviable bacteria. The LPS (endotoxin) used for determination of antibody titers in the study participants was prepared from compost obtained from the Camden and Beltsville-Blue Plains compost sites. Levels of specific IgG antibody directed against LPS prepared from Beltsville-Blue Plains compost were significantly higher in the Beltsville and Blue Plains compost workers than in the nonexposed controls in the Washington, D.C. area. No difference in specific IgG antibody levels directed against LPS prepared from Camden compost was observed in exposed and unexposed workers in the Camden, NJ area. The immune response observed in the Blue Plains-Beltsville compost workers to challenge with LPS by the respiratory route was similar to that reported by Rylander et al. for workers in cotton mills exposed to gram-negative bacteria in cotton dust and rats challenged with purified *E. coli* 025:B6 LPS (14,15).

BEGIN
LAST LINE
OF TEXT

OPTIONAL
SPACE DATA
ENTERED
HERE FOR
TABLES
AND FIGURES

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
PAGE
AREA

CHOPPED
HEAD
SECTION
HERE

Preliminary analysis of other parameters studied, such as C-reactive protein, total immunoglobulin (IgG, IgM, IgA, IgE) levels, liver function tests, skin test reactivity to *A. fumigatus*, chest X-rays and absolute eosinophil counts, did not reveal significant differences among the three exposure groups. When the available data is subjected to a more complete analysis, incorporating such factors as age, race, precise length of exposure, the preliminary findings may require revision. In addition, an extended period of observation may reveal abnormalities which are related to the length of exposure. The preliminary data on antibody response of the compost workers at the Beltsville-Blue Plains sites to LPS suggest that it may be advisable to take reasonable precautions to reduce exposure such as the use of respirators by compost workers and periodic water spraying of the compost sites to reduce dust.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
PAGE AREA
OUTSIDE
CHOPPED
HEAD TABLE
AND ILLU-
STRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
PAGE
HERE

INSERT
HEAD-
ING OR
SECTION
HERE

SECTION 5

AN ENVIRONMENTAL HEALTH SURVEY OF DRINKING WATER CONTAMINATION BY LEACHATE FROM A PESTICIDE WASTE DUMP - HARDEMAN COUNTY, TENNESSEE

Toxic waste dumps are a major source of environmental pollution in many areas of the country and are being recognized with increased frequency. In Hardeman County, Tennessee, wastes from a Memphis, Tennessee, pesticide manufacturer have contaminated the groundwater aquifer serving about 60 nearby residents. During the years of operation, 1964-1972, an estimated 300,000- 500,000 barrels of liquid and solid waste were buried in shallow trenches dug into the ridges at the approximately 200 acre dump site. The dump was closed by state authorities in 1972 because contamination had been detected in test wells close to the burial areas (32,33). The analyses performed at that time did not reveal any contamination in the three private water wells closest to the dump which were being monitored. However, during the fall of 1977, nearby residents became alarmed upon detection of unusual and unpleasant tastes and odors in their well water. Within a short time, the residents reported an unusually high number of illness symptoms including skin and eye irritation, weakness in the upper and lower extremities, upper respiratory infection, shortness of breath and severe gastrointestinal symptoms including nausea, diarrhea and abdominal cramping. In general, these symptoms appeared to subside once ingestion of the contaminated water was stopped. In an effort to determine the extent and possible etiology of the signs and symptoms of illness, a limited health screening study was conducted by investigators from the University of Cincinnati in November 1978. The results from this study showed biochemical changes suggestive of liver dysfunction in some of the residents whose water was contaminated. A second more extensive evaluation was carried out in January 1979. A 1978 investigation by the U.S. Geological Survey confirmed the residents' observations that contamination had occurred (34).

BACKGROUND

The Hardeman County toxic waste dump first came to the attention of the investigators during the course of a seroepidemiologic research study of wastewater treatment plant personnel in Memphis, Tennessee, which is located about 60 miles from the dump site. One of the two treatment plants involved in this study served an area which included the pesticide manufacturer whose waste had formerly been deposited in the Hardeman County toxic waste dump. Chemical wastes from this manufacturer had apparently been routinely discharged into the Memphis sewers and flowed into the Memphis North wastewater treatment plant beginning with its operation in August 1977. A portion of these wastes became airborne at the treatment plant

BEGIN
LAST LINE
OF TEXT
HERE

BOTTOM OF
PAGE AREA
OUTSIDE
MARGIN
FOR TABLE
AND FIGURE
CAPTIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
PAGE
HERE

DISCARD
HEAD
SECTION
HERE

because of the aeration process utilized and because of the open basins that are generally used in such facilities. Workers at the North Plant began complaining of eye irritation and respiratory distress in the spring of 1978 during periods of time when chemical odors at the treatment plant were unusually intense. In conjunction with the ongoing study at the treatment plant, a urine screening study of the workers was conducted in May and June 1978, and air samples were collected at various locations in the treatment plant. Hexachlorocyclopentadiene (HEX) and hexachlorobicycloheptadiene (HEX-BCH), two compounds produced by the pesticide manufacturer, were detected in air, wastewater and urine specimens obtained at that time. In the June testing workers at the Memphis North Treatment Plant were found to have an increased concentration of the HEX-BCH compound in comparison to urine specimens collected early and late in the work shift (35). Because the same chemical company was involved in the Memphis sewage treatment plant situation and in the disposal of toxic waste at the landfill, an effort was made to learn whether any exposure or ill health effects had been detected in the community whose well water was contaminated.

The U.S. Environmental Protection Agency and the State of Tennessee, by the late part of 1978, had detected more than one dozen chlorinated organic compounds in the wells of individuals near the dump site (36). Many of these chemicals (Table 11) were on the National Priority Pollutant Consent Decree List (37). Carbon tetrachloride was the most abundant contaminant detected. Two factors were instrumental in bringing about the health study which began in November 1978. First of all, on investigation of this problem, it became apparent that no health study had been conducted nor was one planned for the near future involving the users of this contaminated water. Second, because of the presence of pesticide compounds in the sewage treatment plant workers' urine specimens and their symptomatology, it was felt appropriate to expand the study to include residents whose wells were contaminated with some of the same compounds.

METHODS AND PROCEDURES

The November 1978 survey of the people using the contaminated water was planned and executed over a short period of time. Shortly after it became known that a wide variety of organic chemicals were contaminating the drinking water, an order or directive suggesting that the individuals concerned stop consuming the water, came from the Environmental Protection Agency. The investigators, therefore, hoped to evaluate the residents while any physical or biochemical changes were still evident. Because of this short time and because of limited personnel, the study was designed to include: 1) a questionnaire that could be administered by the public health nurses involved in the study, including alcohol and drug intake as well as past history of hepatitis or other liver abnormalities, 2) a blood specimen for determination of liver profile, 3) urinalysis for use in detecting the presence of selected organic chemicals, and finally, 4) analysis for selected organic compounds in air and water samples collected from some of the homes of those residents whose wells had been found to be affected. This study was conducted primarily to ascertain whether or not these chemicals, many of which are known hepatotoxins, would cause liver dysfunction in the concen-

DISCARD
LAST LINE
OF TEXT

DISCARD
DATE AREA
OUTSIDE
MARGINS
FOR TABLES
AND LARGE
FIGURES

TABLE 11. CONTAMINANTS DETECTED^a IN PRIVATE WELLS IN TOONE-TEAGUE
AREA OF HARDEMAN COUNTY, TENNESSEE

Compound	Highest level detected (ppb)
Benzene ^b	15
Carbon Tetrachloride ^b (CCl ₄)	18,700
Chlordene	0.81
Chlorobenzene ^b	41
Chloroform ^b (CHCl ₃)	1,890
Hexachlorobutadiene ^b	2.53
Hexachloroethane ^b	4.6
Hexachlorobicycloheptadiene (HEX-BCH)	2.2
Methylene Chloride ^b	160
Napthalene ^b	6.7
Tetrachloroethylene ^b	2,405
Toluene ^b	52
Xylenes	1.6

^aAdapted from U.S. Environmental Protection Agency, Region IV, March 9, 1979, Summary of USEPA and State of Tennessee Chemical Analyses (36).

^bOn National Priority Pollutants Consent Decree List (37).

trations at which they were found. These results were compared to a control group from Memphis which had been collected two months earlier.* The location of the affected wells in relation to the dump site is shown in Figure 1.

The blood specimens that were drawn were centrifuged and the serum was frozen and shipped to Cincinnati for performance of routine liver function determinations including serum gamma glutamic transaminase (SGGT), serum glutamic oxalic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase and total bilirubin. These tests were performed in the Clinical Laboratory of Cincinnati General Hospital. The Hardeman County specimens were analyzed in November and the Memphis specimens in September and January. The urine specimens and water samples were

*The November 1978 results were later compared to those from a local control group obtained in January 1979.

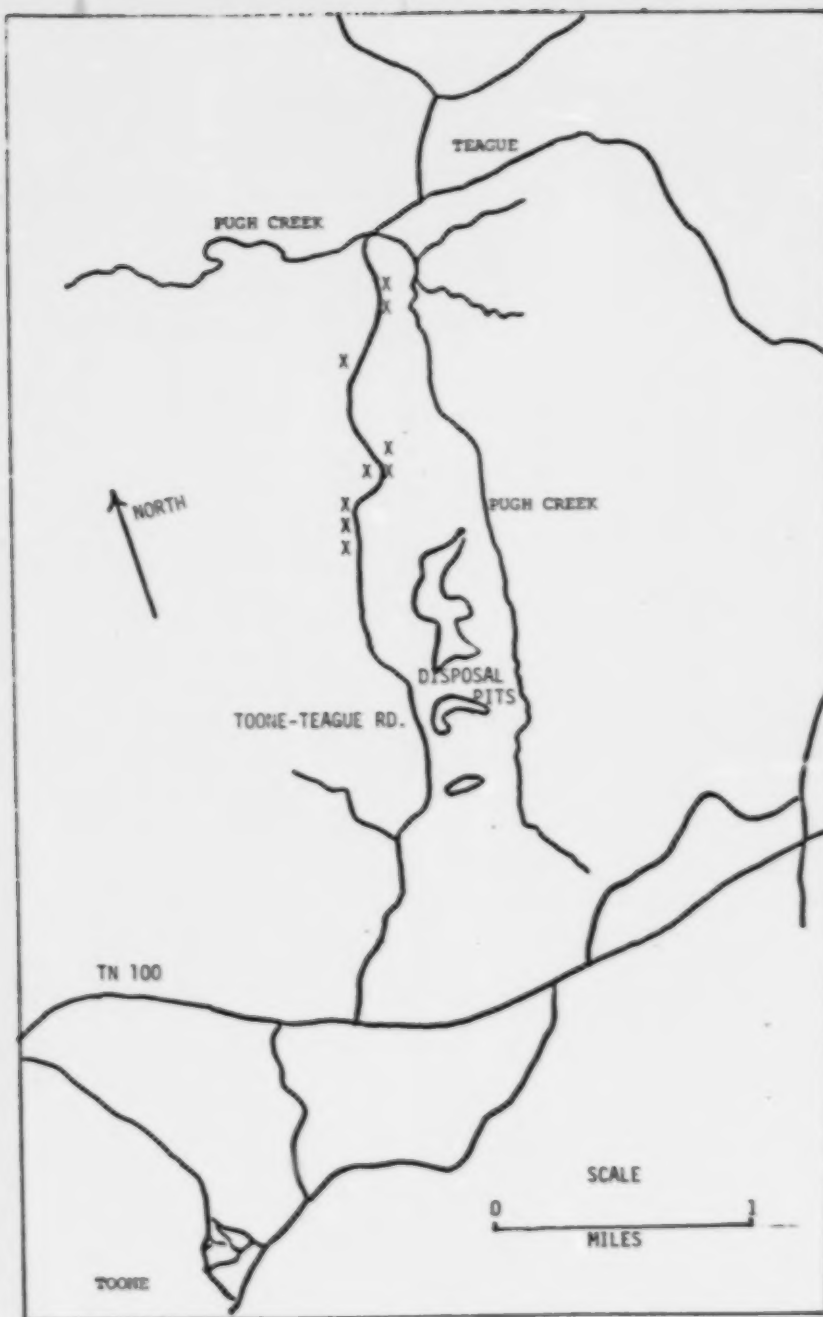


Figure 1. Location of contaminated private wells near Hardeman County toxic waste dump (designated by X).

frozen immediately after collection and an aliquot of each was saturated with sodium chloride and extracted with equal volume of petroleum ether. The petroleum ether extracts were stored in a freezer until time of analyses.

The air samples were collected using glass sampling tubes containing 150 mg of preextracted chromosorb 102 and desorbed with petroleum ether. All of these specimens were analyzed by electron capture gas chromatography using glass columns, packed with OV-10 or OV-17-QF1, at 175°C and were performed at the laboratories of the Department of Environmental Health at the University of Cincinnati, College of Medicine, Cincinnati, Ohio.

The statistical analyses of the liver function tests were performed on the controls using age, sex, and a factor for maturity level as variables; covariant adjustments from this analysis were then used in analysis of variance for the entire group of data.

When the preliminary results from the initial study were analyzed, it was apparent that there were serum enzyme levels suggestive of liver dysfunction present in serum of those consuming the contaminated water. As a result, a decision to expand the November 1978 study was made and this was conducted in January of 1979. The follow-up study consisted of a more detailed questionnaire including past medical history, alcohol intake, drug consumption, history of hepatitis and other liver abnormalities, as well as general health survey questions. In addition to this, a clinical examination was performed to assess blood pressure, liver size and other physical parameters. Blood was obtained while fasting for determination of serum bile acids, a liver profile, a renal profile and the hepatitis serology. The hepatitis tests were included to evaluate whether any differences in liver enzyme tests were related to previous infection. Postprandial specimens were collected two hours after a breakfast consisting of eggs, bacon or sausage, juice, toast and coffee or milk for analyses for serum and urine bile acids. The study was conducted at the Chickasaw State Park, located about 10 miles from the dump site. The study group was expanded to include not only area residents whose wells were contaminated but a number of other people from local civic organizations and church groups were invited to participate as controls who lived in the same geographic area as those whose water supplies were contaminated. There was also another group which included those who were frequent visitors to the households of those persons with contaminated water or who were close neighbors whose water was not significantly contaminated and were considered to represent an intermediate exposure population. A map showing the location of the residences of the controls recruited for the January 1979 study and their relation to the toxic waste dump is shown in Figure 2. Persons with abnormal liver function tests in January 1979 had repeat determinations performed on serum collected in March 1979.

Participants who had occupational exposure to pesticides, who had consumed alcohol 24 hours prior to the collection of the blood specimens or who had a history of a liver disorder were deleted from the analysis of liver and renal profiles and serum and urine bile acids. The dependent variables were then tested for normality and transformations were made when necessary.

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
PAGE
LINE

INCHES
HEAD
BORN
SECTION
HERE

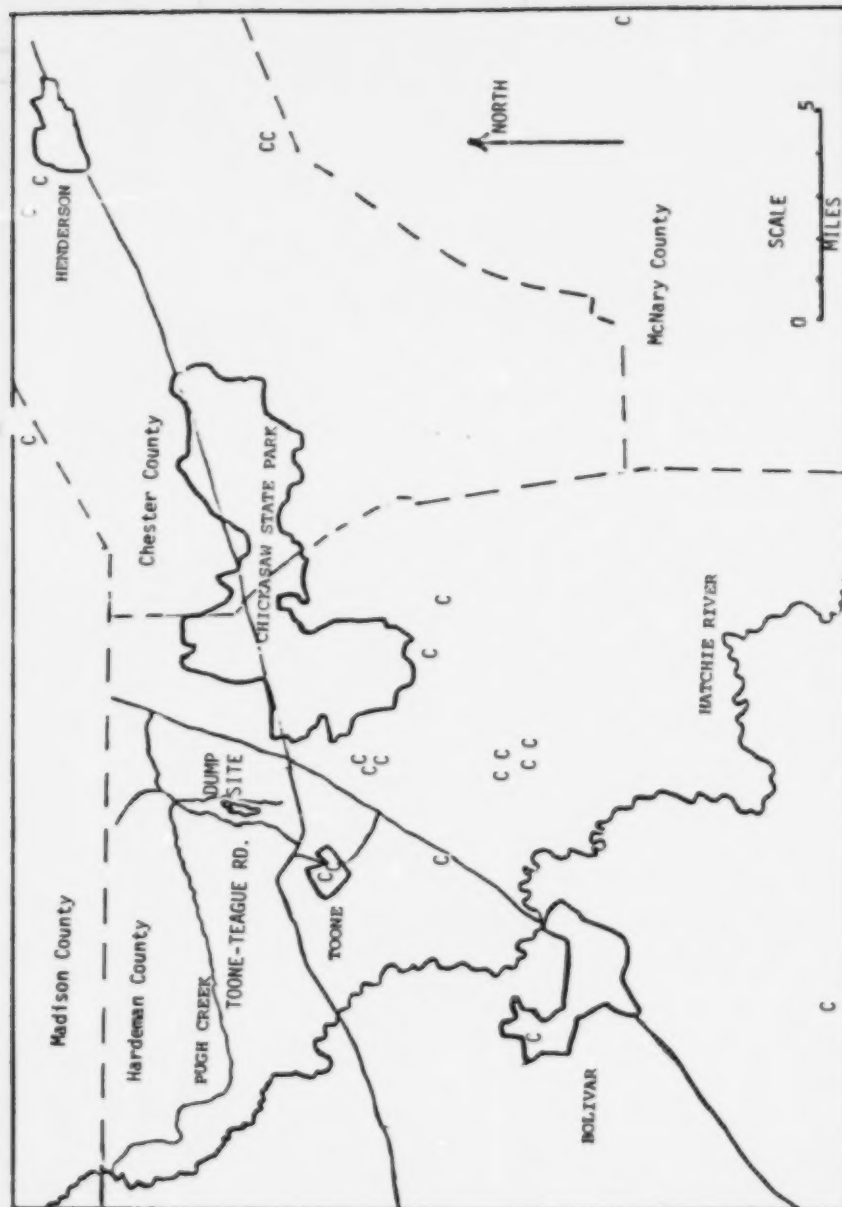


Figure 2. Location of homes of January 1979 control group in relation to dump site (designated by letter C).

BEGIN
LAST LINE
OF TEXT
HERE

OPTIONAL
MARGIN
DIMENSIONS
FOR TABLES
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

CHANGED
HEAD
SECTION
HERE

Analysis of covariance was used to assess the effects of age, sex, monthly alcohol consumption and their interactions on the dependent variables of the controls. Adjustments were then made for those covariables which were found to be significant. One-way analysis of variance was used to test whether there were significant differences between the exposed, intermediate-exposed, and control groups in the January 1979 study. When all three groups were tested simultaneously, analysis of variance with 2 degrees of freedom was used. Since this analysis involved multiple comparisons, the significance level was set at 0.02. The exposed group from the November 1978 study was also compared to the control group from the January 1979 study.

Representative air and water samples were collected from residences of the exposed, intermediate-exposed and control groups. In some cases, indoor air samples were collected in bathrooms with either the bathtub or the shower tap running.

In order to test whether the 30 people in the study group who were tested in both November 1978 and January 1979 showed a significant change in their hepatic profile, the differences between the results of the two periods were analyzed. These differences were found to be normally distributed except for SGGT which required a log transformation of its subtraction from a constant. A t-test was then performed on the differences.

Results - November 1978

Water Use--

During the period from May to December 1978 a trailer-mounted water tank served as a temporary supply for kitchen and drinking uses. Periodically the tank was refilled in a nearby community. A second temporary supply piped from an underground holding tank to a number of the residences was installed in December 1978. A permanent water supply from the village of Toone was connected to most of the residents in 1979. On the day of the collection of blood and urine from Hardeman County residents, many of the people in the affected area had received certified letters from the U. S. Environmental Protection Agency (Region IV) advising them not to use the water for any purposes because of high amounts of carbon tetrachloride found. Some of the residents had stopped using the water for potable uses (drinking and cooking) beginning as early as the fall of 1977. A large group stopped such use in May 1978 after being advised by public agencies that the water was unsafe. By August 1978 practically all had ceased potable use of the contaminated water. Thus, any abnormalities reflected by the specimens collected in this survey would for the most part be due to current exposure from washing and toilet uses and prior exposure from drinking and cooking.

Population Characteristics--

A total of 36 exposed Hardeman County people were available for comparison with a total of 53 people in the Memphis control group. These groups were composed of 14 males and 22 females from Hardeman County, and 23 females and 30 males from Memphis. All participants were white. An age and sex

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSIONS
FOR TABLES
AND ILLUSTRATION

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

profile for the two groups is shown in Table 12. Four additional Hardeman County residents provided urine specimens only.

Liver Function Tests--

The results of the comparisons of the Hardeman County and Memphis group are presented in Tables 13 and 14. Significant differences were found for several of the tests, alkaline phosphatase, SGPT and SGOT levels were significantly higher in the Hardeman County specimens ($p = .017$, $.039$ and $.0001$, respectively). Albumin and total bilirubin were lower in the Hardeman County population ($p = .0002$ and $.0001$, respectively). Examination of the variation of results for age and sex (Table 14) revealed that for SGOT the geometric mean for Hardeman County males, 22.0, was at the upper limit of the normal reference range of 8-22. The geometric mean for Hardeman County persons under 12 years of age was 23.4 was slightly above the limit for the normal range and that for persons 12-21 years was slightly below this limit, 21.6.

Analysis of Urine Samples for Selected Organic Compounds--

In November 1978, urine specimens from 96 participants (43 exposed Hardeman County residents and 53 Memphis controls) were screened for five chlorinated organics HEX, HEX-BCH, heptachlorobicycloheptene, chlordene and heptachlor. None of the samples from the Hardeman County residents showed the presence of any of these substances. However, urine from six of the adult males in Memphis contained HEX-BCH in amounts ranging from 0.3 to 1.0 ng/ml, probably due to the proximity of their location of work to the pesticide manufacturer.

Environmental Data--

Environmental data from air and water samples collected from several locations in the two communities are presented in Tables 15 and 16. Air and water samples were not taken in the homes of the Memphis families used in these comparisons. However, such sampling was performed elsewhere in Memphis at about the time of the collection of their blood and urine specimens. Of particular interest is the air data for August 23, which was taken in the bathroom of one residence in Hardeman County, the first sample was collected with no water running and the second with the shower on and the door closed. Levels of carbon tetrachloride increased by over a factor of 100 between these two samples. The higher value is approximately five percent of the ACGIH recommended occupational standard for work place air (38). The reduction in tetrachloroethylene (TCE) in the sample taken with the shower running may be due to more effective collection and retention of carbon tetrachloride by the sampling sorbent or displacement of TCE by carbon tetrachloride in the sampling material. The only air sample during this time period which was associated with a noticeable air odor was the August 23, 1978 sample collected with the shower running.

Detectable levels of HEX in air were found in three of the five Hardeman County home samples in November 1978. The highest concentration was $0.10 \mu\text{g}/\text{m}^3$. In comparison, air levels at the Memphis North Treatment Plant ranged up to 39 and $278 \mu\text{g}/\text{m}^3$ for HEX and HEX-BCH, respectively. Concentration of these same two compounds in urine collected from treatment plant workers ranged up to 3.9 and $10.3 \mu\text{g}/\text{L}$, respectively (39).

TABLE 12. AGE AND SEX PROFILE OF HARDEMAN COUNTY EXPOSED STUDY GROUP AND MEMPHIS CONTROL GROUP (NOVEMBER 1978)

Age, Range Years	Location and Sex			
	Hardeman County		Memphis	
	Males	Females	Males	Females
Less than 12 years	5	7	2	6
12-21 years	2	4	5	7
More than 21 years	<u>7</u>	<u>11</u>	<u>23</u>	<u>10</u>
Totals	14	22	30	23

TABLE 13. HEPATIC PROFILE COMPARISON OF HARDEMAN COUNTY EXPOSED GROUP
AND MEMPHIS CONTROL GROUP (NOVEMBER 1978)

Parameter		Hardeman County	Memphis	Significance of Difference (t-test)
Alkaline Phosphatase (32-72 mU/ml adults 21, 25-150 mU/ml children <21)	Mean**	88.1	59.9	.017
	Range	34-360	29-191	
	No. Above Normal	17/36	6/53	
	Total Tested			
Serum gamma glutamic transaminase (SGGT) (5-29 mU/ml)	Mean*	9.47	11.23	.574
	Range	2-54	4-68	
	No. Above Normal	3/36	4/51	
	Total Tested			
Albumin (3.5-5.0 gr/dl)	Mean	4.35	4.53	.0002
	Range	3.9-4.8	3.7-5.7	
	No. Above Normal	0/36	16/49	
	Total Tested			
Total Bilirubin (0.1-1.1 mg/dl)	Mean*	.240	.490	.0001
	Range	0.1-0.8	0.1-2.0	
	No. Above Normal	0/31	3/40	
	Total Tested			
Serum glutamic pyruvic transaminase (SGPT) (5-25 mU/ml)	Mean*	15.9	13.3	.039
	Range	9-50	4-60	
	No. Above Normal	5/36	3/53	
	Total Tested			
Serum glutamic oxalic transaminase (SGOT) (8-22 mU/ml)	Mean*	19.5	14.9	.0001
	Range	12-36	3-35	
	No. Above Normal	11/36	8/51	
	Total Tested			

*Normal range indicated in parenthesis

* *Geometric mean

TABLE 14. VARIATION IN HEPATIC PROFILE WITH AGE AND SEX, HARDEMAN COUNTY - MEMPHIS
COMPARISON (NOVEMBER 1978)

	(Mean Values)					
	Age			Sex		
	Less Than 12 Years		12-21 Years	Males		Females
	Hardeman Co.	Memphis	Hardeman Co. Memphis	Hardeman Co.	Memphis	Hardeman Co. Memphis
Age (N)	(12) 9.1	(8) 3.5	(6) 15.8 (12) 16.9	(14) 26.5	(30) 34.1	(22) 22.7 (23) 22.6
Alk. Phos.* (N)	(12) 187.3	(8) 151.7	(6) 94.6 (10) 63.2	(14) 87.5	(29) 56.4	(22) 88.5 (22) 65.0
SGGT* (N)	(12) 7.28	(8) 5.90	(6) 6.17 (10) 8.87	(14) 10.2	(29) 16.1	(22) 9.04 (22) 7.00
Albumin (N)	(12) 4.35	(8) 5.24	(6) 4.48 (10) 4.97	(14) 4.40	(29) 4.20	(22) 4.31 (22) 4.97
Total Bili.* (N)	(11) 0.21	(3) 0.16	(5) 0.20 (7) 0.40	(12) 0.20	(26) 0.60	(19) 0.27 (14) 0.33
SGPT* (N)	(12) 15.4	(8) 18.2	(6) 19.2 (12) 13.8	(14) 18.8	(30) 14.1	(22) 14.4 (23) 12.4
SGOT* (N)	(12) 23.4	(8) 21.8	(6) 21.6 (12) 13.2	(14) 22.0	(30) 15.2	(22) 18.1 (23) 14.4

*Geometric mean
(N) = Number of individuals in group.

$\mu\text{g}/\text{m}^3$

Sample Location	Date	HEX	HEX-BCH	HEX-VCL *	Chlordene	CCl4	Tetrachloroethylene
<u>Hardeman County Exposed Group</u>							
Residence 1 - bathroom (shower off)	8/23/78	.07	.05	n.d.	n.d.	23	302
Residence 1 - bathroom (shower on)	8/23/78	n.d.	0.5	n.d.	n.d.	3600	120
Residence 1 - kitchen	11/6/78	0.10	n.d.	n.d.	n.d.	3.6	3.6
Residence 2 - bathroom **	11/6/78	.09	n.d.	n.d.	n.d.	10	2.4
Residence 3 - living room	11/6/78	n.d.	n.d.	n.d.	n.d.	8.8	2.5
Residence 4 - kitchen	11/6/78	.06	n.d.	0.6	n.d.	36	5.7
Residence 5 - living room	11/6/78	n.d.	n.d.	n.d.	n.d.	41	n.d.
<u>Other:</u>							
Memphis Residences	9/5-9/8/78	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.
Memphis Industrial Area ***	9/6/78	n.d.	0.05	0.3	n.d.	n.a.	n.a.
Memphis Library	9/13/78	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.
Memphis Residence	11/6/78	n.d.	n.d.	n.d.	n.d.	1.2	2.7

n.d. = none detected

n.a. = not analyzed

- Heptachlorobicycloheptene

** Also contained heptachlor (2.2 ng/l)

*** located near pesticide manufacturer

TABLE 16. CONCENTRATIONS OF SELECTED ORGANIC COMPOUNDS IN WATER SAMPLES (1978)

Sample Location	Date	$\mu\text{g/l}$					CCl ₄
		HEX	HEX-BCH	HEX-VCL	Chlordene		
<u>Hardeman County Exposed Group</u>							
Residence 1	10/2/78	0.72	0.25	n.d.	n.d.	n.a.	n.a.
Pugh Creek	10/2/78	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.
Residence 1	11/7/78	n.d.	0.06	n.d.	n.d.	618	
<u>Other:</u>							
Three residences (two in Memphis and one in Bolivar, TN)	10/2/78	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.

n.d. = none detected

n.a. = not analyzed

Medical Histories--

Medical questionnaires focused on illnesses during the 12 month period prior to the study, revealed that there were 11 hospitalizations of the Hardeman County residents. Symptoms included gastrointestinal abnormalities, respiratory difficulties, neurological symptoms, muscular complaints, and high fever. Many other persons reported becoming ill with symptoms such as dizziness, headache, skin rashes, nausea and vomiting, numbness of limbs, and menstrual irregularities. During this same time period the only birth was a low birth weight infant with gastrochisis and eventration of intestines. Seven adults and five children reported vision problems.

Summary

This survey revealed that concentrations of the enzymes alkaline phosphatase, SGPT and SGOT were statistically higher in the residents who drank the contaminated water group than in the control group. This difference existed even though the adult males from the Memphis control group were exposed to some air contamination by the "HEX compounds" as revealed by the air sample from a Memphis industry (Table 15) and by the presence of HEX-BCH in some of the specimens. The differences appear to be associated with well water contaminated by the toxic waste landfill. Residents and others have noticed chemical-like odor emanating from the dump site during the period of its operation and on more recent occasions indicating that the exposure undoubtedly was not due entirely to the direct contact with contaminated water. Based on the results of the November 1978 survey it was also concluded that a more extensive survey was warranted.

Results - January 1979

Water Use--

At the time of this study all persons in the exposed group were using the temporary piped water supply. Two families in the intermediate-exposure group were using well water containing about 50 ppb of carbon tetrachloride (36). Carbon tetrachloride was not detected in any other water supplies tested (see Environmental Monitoring Results).

Population Characteristics--

The age and sex distribution for the exposed, intermediate-exposed and control groups are presented in Table 17.

Liver Function Tests--

Results of comparisons between the January 1979 study groups appear in Table 18. There were no significant differences detected between groups for the liver function tests. The 30 people who were tested in both November 1978 and January 1979 showed a statistically significant change for all six variables with alkaline phosphatase, SGGT, SGPT and SGOT all decreasing from November 1978 to January 1979 while albumin and total bilirubin increased (Table 19). The mathematical transformations used in the statistical analyses of liver function test data and the significant covariables are presented in

TABLE 17. AGE AND SEX PROFILE OF EXPOSED, INTERMEDIATE-EXPOSED
AND CONTROL GROUPS IN HARDEMAN COUNTY STUDY
(JANUARY 1979)

Age	Exposed		Intermediate-Exposed		Control	
	Male	Female	Male	Female	Male	Female
0-14	6	13	3	12	4	7
15-24	2	3	0	2	1	4
25-34	5	8	5	7	7	10
35-44	3	1	0	0	3	4
45-54	0	1	0	0	2	2
55-64	3	1	1	1	1	7
65 and over	1	2	1	1	1	3
Total	20	29	10	23	19	38*

*One missing age

TABLE 18. HEPATIC PROFILE COMPARISON OF HARDEMAN COUNTY EXPOSED,
INTERMEDIATE-EXPOSED AND CONTROL GROUP (JANUARY 1979)

		Groups		
		Exposed	Intermediate-exposed	Control
Alkaline phosphatase	Mean	76.33	79.02	61.49
	Range	29-450	34-186	31-220
	No. above normal	18/48	15/33	8/56
	Total tested			
SGGT	Mean	12.27	10.32	11/56
	Range	5.51	5-35	4-56
	No. above normal	6/48	1/33	3/56
	No. tested			
Albumin	Mean	4.80	4.81	4.93
	Range	3.0-7.6	4-5.6	4.2-6.2
	No. above normal	16/48	9/32	23/57
	Total tested			
Total bilirubin	Mean	0.49	0.51	0.51
	Range	0.2-1.4	0.2-1.6	0.2-1.7
	No. above normal	4/47	3/28	4/52
	Total tested			
Direct bilirubin*	Mean	0.12	0.12	0.13
	Range	0.1-0.2	0.1-0.2	0.1-0.2
	No. above normal	0/47	0/26	0/52
	Total tested			
SGPT	Mean	12.45	14.08	14.25
	Range	2-49	7-27	6-70
	No. above normal	1/48	1/33	4/56
	Total tested			
SGOT	Mean	16.91	16.31	16.08
	Range	10-41	11-25	9-140
	No. above normal	11/48	5/33	7/56
	Total tested			

*Normal range 0.1 - 0.4 mg/dl

TABLE 19. COMPARISON OF HEPATIC PROFILE TEST RESULTS FOR EXPOSED PARTICIPANTS IN BOTH NOVEMBER 1978 AND JANUARY 1979 STUDIES

Variable	N	Mean Difference *	Standard Error	t	Degree of Freedom	p
<u>Hepatic Profile:</u>						
Alkaline phosp.	31	13.3	6.3	2.125	30	$p < .0419$
SGGT (log (5-SGGT))	31	-2.6	1.1	2.302	30	$p < .0284$
Albumin	31	-.5	.13	3.922	30	$p < .0004$
Total bilirubin	26	-.2	.03	6.405	25	$p < .0001$
SGPT	31	3.6	1.2	3.044	30	$p < .0048$
SGOT	31	4.4	1.0	3.989	30	$p < .0003$

*November results minus January results.

Table 20 along with similar information for the bile acid and renal function test data.

Bile Acids--

Results of analyses of fasting and nonfasting serum and urine samples for specific bile acids - sulfated conjugates of lithocholate (SLCC) and cholyglycine (CG) appear in Table 21. The mean nonfasting serum SLCC values were significantly lower in the exposed group than in the control, however, the clinical significance of this finding is unknown, since SLCC increases in most liver diseases.

The mean change in SLCC and CG from the fasting to the nonfasting serum specimens was positive for all groups as expected, however, in urine it was negative for the exposed group and positive for the other two. These changes in urine CG were statistically different for the exposed and control groups.

Bile acid determinations were also made on the nonfasting serum samples collected from the exposed group in November 1978. Twenty-two of these individuals were retested in January 1979. A comparison of the November 1978 and January 1979 analyses (Table 22) revealed that serum concentrations for SLCC were significantly higher in November 1978 than in January 1979.

Renal Function Tests--

Results of comparisons between the January 1979 exposed and control groups appear in Table 23. Differences were found for total carbon dioxide with the exposed group having somewhat lower total carbon dioxide levels than the control group. Renal function tests were not performed on the intermediate-exposed group.

Hepatitis Serology--

In order to determine if the results of the tests of liver function were due to a prior history of infection with hepatitis, antibodies to hepatitis A and hepatitis B were determined. A log-linear model, taking age into account, was used to determine that the incidence of positive hepatitis serology was not significantly different between the groups.

Physical Examination--

During the physical examination 7 individuals were found to have borderline enlargement of their livers, 6 being in the exposed group and 1 in the intermediate group. Discriminant analysis was used to determine whether there were differences in liver enlargement among the exposed, intermediate-exposed and control groups. A significant difference between the groups was found.

Illness--

Study participants were asked if they had any "serious illnesses" in the prior six months. The subjective complaints were classified as either respiratory, gastrointestinal or renal. Comparison of the results of the illness interviewing (Table 24) indicates borderline differences for respiratory illnesses ($p = 0.08$) and all illnesses combined ($p = 0.06$). In each case the intermediate-exposed groups had a higher illness rate than the exposed groups which had slightly higher rates than the control groups.

TABLE 20. TRANSFORMATION AND SIGNIFICANT COVARIABLES IN THE STATISTICAL ANALYSES OF LIVER FUNCTION, BILE ACID AND RENAL FUNCTION TEST DATA

Variable	Transformation Used	Significant Covariables
<u>Liver Function Tests</u>		
Alkaline phosphatase	log	age
SGGT	log	age, sex
Albumin	log	--
Total bilirubin	log	age, sex, age-sex interaction
Direct bilirubin	log	--
SGPT	log	sex
SGOT	log log	sex
<u>Serum Bile Acids</u>		
SLCC-fasting	log	--
SLCC-nonfasting	log	--
SLCC-difference	--	--
CG-fasting	square root square root	--
CG-nonfasting	square root	--
CG-difference	--	--
<u>Urine Bile Acids</u>		
SLCC-fasting	square root	--
SLCC-nonfasting	square root	--
SLCC-difference	--	age, sex, age-sex interaction
CG-fasting	log	--
CG-nonfasting	square root	--
CG-difference	--	sex
<u>Renal Function Tests</u>		
Na	square	--
K	log	age, monthly alcohol consumption, age-alcohol consumption interaction
CL	square root	--
Total CO ₂	--	--
Glucose	--	age
BUN	log	age
Creatinine	--	age, sex

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD.
BEGIN
SECTIONS
HERE

TABLE 21. COMPARISON OF MEAN BILE ACID LEVELS (+ STANDARD ERROR) IN FASTING AND NONFASTING SERUM AND URINE SAMPLES COLLECTED FROM PARTICIPANTS IN JANUARY 1979 STUDY

Serum Bile Acids	Groups					
	Exposed		Intermediate-Exposed		Control	
	Mean	Standard Error	Mean	Standard Error	Mean	Standard Error
SLCC-fasting	0.769	.091 (48)	0.878	0.136 (29)	0.843	.065 (59)
SLCC-nonfasting	1.106	.078 (42)	1.490	0.220 (24)	1.526	.109 (58) ^A
SLCC-difference	+	.071 (42)	+	.157 (24)	+	.620 (58)
CG-fasting	0.268	.046 (48)	.255	0.053 (29)	0.382	.062 (59)
CG-nonfasting	1.229	.135 (42)	1.081	0.133 (24)	1.277	.102 (57)
CG-difference*	+	.123 (42)	+	.157 (24)	+	.104 (57)
Urine Bile Acids:						
SLCC-fasting	1.607	.209 (41)	1.781	.216 (28)	1.825	.187 (56)
SLCC-nonfasting	1.513	.181 (44)	1.712	0.273 (24)	2.047	.171 (51)
SLCC-difference	-	.155 (38)	+	.204 (24)	+	.135 (50)
CG-fasting	.660	.095 (44)	.635	.140 (28)	0.521	.070 (55)
CG-nonfasting	.645	.074 (44)	0.766	.126 (25)	0.804	.072 (54)
CG-difference*	-	.101 (41) ^B	+	.082 (25)	+	.063 (52) ^B

Values in parenthesis are number of observations.

Letters depict significance between groups at $p \leq 0.02$.

*Nonfasting result minus fasting result.

SLCC - sulfated conjugates of lithocholate

CG - cholyglycine

BOTTOM OF
IMAGE AREA.
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

BEGIN
LAST LINE
OF TEXT

TABLE 22. COMPARISON OF SERUM BILE ACID LEVELS IN NOVEMBER 1978
SAMPLES WITH THOSE IN SERUM SAMPLES COLLECTED
FROM THE SAME INDIVIDUALS IN JANUARY 1979

Serum bile Acids:	N	Mean Difference*	Standard Error	t	Degrees of Freedom	p
SLCC-nonfasting (log (SLCC + 1.5))**	23	0.22	0.09	2.475	22	$p < .0211$
CG-nonfasting	23	0.32	0.23	1.401	22	$p = .1745$

*November results minus January results.

**For SLCC the data required a log transformation of the serum concentration plus a constant in order to produce a normal distribution.

TABLE 23. COMPARISON OF MEAN VALUES (+ STANDARD ERROR) OF RENAL PROFILE TEST RESULTS BETWEEN EXPOSED AND CONTROL GROUPS PARTICIPATING IN JANUARY 1979 STUDY

Renal Profile*	Groups			
	Exposed		Control	
	Mean	Standard Error	Mean	Standard Error
Sodium (Na) (133-145 mEq/l)	141.97 $\pm$.51	(35)	141.26 $\pm$.53	(42)
Potassium (K) (3.5-5.0 mEq/l)	4.606 $\pm$.077	(35)	4.54 $\pm$.07	(42)
Chlorides (Cl) (95-110 mEq/l)	103.50 $\pm$.38	(32)	104.59 $\pm$.54	(41)
Total Carbon Dioxide (Total CO ₂) (21-27 mEq/l)	23.13 $\pm$.96	(32) ^A	26.27 $\pm$.64	(41) ^A
Glucose (60-95 mEq/l)	77.68 $\pm$ 2.35	(34)	82.05 $\pm$ 1.77	(42)
Blood Urea Nitrogen (BUN) (5-17 mg/dl)	13.38 $\pm$.75	(34)	16.48 $\pm$.93	(42)
Creatinine (0.7-1.4 mg/dl)	0.72 $\pm$.03	(34)	0.84 $\pm$.03	(42)

Letter depict significant differences between groups (≤ 0.02).

*Normal ranges indicated in parenthesis.

TABLE 24. COMPARISON OF REPORTED SERIOUS ILLNESSES IN PRIOR SIX MONTHS
(PERCENT SHOWN IN PARENTHESIS)

	<u>Groups</u>		
	Exposed	Intermediate-Exposed	Control
<u>Respiratory</u>			
Number with illness	6(11)	10(23)	5(8)
Number without illness	47(89)	33(77)	56(92)
$\chi^2 = 5.23, p = 0.08$			
<u>Gastrointestinal</u>			
Number with illness	3(6)	4(9)	3(5)
Number without illness	50(94)	39(91)	58(95)
$\chi^2 = 0.88, p = 0.65$			
<u>Renal</u>			
Number with illness	1(2)	1(2)	2(3)
Number without illness	52(98)	42(98)	59(97)
$\chi^2 = 0.23, p = 0.88$			
<u>All Illnesses Combined</u>			
Number with illness	10(19)	13(30)	7(12)
Number without illness	43(81)	30(70)	54(88)
$\chi^2 = 5.74, p = 0.06$			

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

Extensive review of the medical histories obtained at the time of the indepth investigation as well as review of hospital records supplied by patients who had undergone hospitalization in the last several years was performed. Hospital records showed no indication of any disease trend patterns or clustering of diseases that could be attributed to consumption of contaminated water. Many residents, however, did report nausea, vomiting and mucous membrane irritation and associated these symptoms with an unusual chemical-type odor in the water used for consumption.

DROPPED
HEAD,
BEGIN
SECTIONS
HERE

Analysis of Urine Samples for Selected Organic Compounds--

Urine specimens from 137 participants from the three groups of participants were screened for the presence of HEX, HEX-BCH, hexachlorobicycloheptene, chlordene and heptachlor. None of the samples showed the presence of the selected chlorinated organics. A select group of 9 individuals judged to have relatively high potential for exposure to carbon tetrachloride were sampled for determination of carbon tetrachloride in urine. Of the 9 persons, 5 were from the exposed group and 4 from the intermediate-exposed group. These intermediate-exposed participants included individuals who were using water containing about 50 $\mu\text{g}/\text{l}$ of carbon tetrachloride. No evidence was found for carbon tetrachloride in any of these urine specimens. These findings with human urine specimens is consistent with animal studies (40,41) which show that carbon tetrachloride administered to mammals is largely excreted through the lungs, that only a small fraction of absorbed material is metabolized, and that it is not excreted as the parent compound in the urine. The primary metabolites include chloroform, hexachloroethane, and carbon dioxide. It was, therefore, concluded that analysis of additional urine specimens for carbon tetrachloride was not warranted at this time.

Environmental Monitoring--

Representative water samples were analyzed from all of the sources of domestic water for the homes of the study participants. The monitoring was conducted in both January and March 1979. The March monitoring included some of the same homes as those sampled in January. For a number of the homes indoor and/or outdoor air samples were collected.

Tables 25 and 26 provide a comparison of air and water samples collected in January 1979 and March 1979, respectively for the exposed, intermediate-exposed and control groups. Water samples were designated as temporary (T), individual wells (I) and public (P). A temporary water supply was in use for many homes along the Toone-Teague Road including all homes of exposed participants and some homes of the intermediate-exposed participants. Inside air samples were collected in the bathrooms with the shower or bathtub tap running. The water samples containing the highest concentrations of carbon tetrachloride and tetrachloroethylene were from the group one and two homes. In general, the samples from the control group had typical background levels (42,43). Of the six control homes using a public water supply one had an inside air sample containing greater than 1 $\mu\text{g}/\text{m}^3$ of carbon tetrachloride. Low levels of carbon tetrachloride, up to 1.1 $\mu\text{g}/\text{L}$, were detected in some of the public water supply samples which were routinely chlorinated.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLE
AND ILLUSTRATIONS

TABLE 25. CONCENTRATION OF SELECTED ORGANIC COMPOUNDS IN WATER SAMPLES AND AIR SAMPLES FOR RESIDENCES OF STUDY PARTICIPANTS (JANUARY 1979)^a

Group and sample	Type of Water Supply ^b	Water Concentration $\mu\text{g/L}$	Air Concentration $\mu\text{g/m}^3$ Outside ^c	Inside ^c
Carbon Tetrachloride				
Exposed				
R-1 ^d	T	ND	74.6	0.6
R-6	T	ND	0.5	6.3
Intermediate-Exposed				
R-7	I	ND	0.4	176
R-8	I	ND	NS ^e	NS
R-9 ^f	I	ND	NS	NS
R-10	I	3.7	NS	NS
R-11	I	0.7	NS	NS
R-12	I	ND	NS	NS
R-13	I	ND	NS	NS
Control				
R-14	I	ND	NS	NS
R-15	I	ND	0.2	ND
R-16	I	ND	NS	NS
R-17	I	ND	0.5	0.2
R-18	I	ND	NS	0.3
R-19	I	ND	1.1	NS
R-20	P	ND	0.1	0.4
R-21	P	ND	0.6	0.2
R-22	P	ND	0.2	ND
R-23	P	ND	NS	NS
R-24 to R-339	I	ND	NS	NS
Tetrachloroethylene				
Exposed				
R-1	T	ND	0.4	1.2
R-6	T	ND	ND	1.0
Intermediate-Exposed				
R-7	I	ND	0.3	11.6
R-8	I	ND	NS	NS
R-9	I	ND	NS	NS
R-10	I	ND	NS	NS
R-11	I	ND	NS	NS
R-12	I	ND	NS	NS
R-13	I	ND	NS	NS

(continued)

TABLE 25 (continued)

Group and sample	Type of Water Supply ^b	Water Concentration $\mu\text{g/L}$	Air Concentration $\mu\text{g/m}^3$	
			Outside ^c	Inside ^c
Control				
R-14	I	ND	NS	NS
R-15	I	ND	0.3	0.3
R-16	I	ND	NS	NS
R-17	I	ND	0.3	0.4
R-18	I	ND	NS	0.6
R-19	I	ND	0.5	NS
R-20	P	ND	0.2	0.2
R-21	P	ND	0.2	0.2
R-22	P	ND	0.2	0.2
R-23	P	ND	NS	NS
R-24 to R-33	I	ND	NS	NS

^aAll samples were analyzed for: hexachlorocyclopentadiene (HEX), hexachlorobicycloheptadiene (HEX-BCH), heptachloronorborene, chlordane, heptachlor, carbon tetrachloride and tetrachlorethylene.

^bThe types of water supplies in use were: T - temporary supply provided for homes with contaminated wells; I - individual wells; and P - public supply.

^cInside samples were collected in a bathroom with water running. Outside samples were collected within 10 meters of the house.

^dThe outside air sample also showed $3.2 \mu\text{g/m}^3$ of HEX-BCH.

^eNS - indicates that no sample was obtained.

^fThis household was using a hand carried water supply, source unknown, for potable purposes.

^gWater samples only were obtained from these ten residences in the control group. None of the substances analyzed in this study were found in any of these samples.

TABLE 26. CONCENTRATION OF SELECTED ORGANIC CHEMICALS IN WATER AND AIR
SAMPLES FOR RESIDENTS OF STUDY PARTICIPANTS (MARCH 1979)^a

Group and sample	Type of Water Supply ^b	Water Concentration	Air Concentration $\mu\text{g}/\text{m}^3$ ^c	
		$\mu\text{g}/\text{L}$	Outside	Inside
Exposed		Carbon Tetrachloride		
R-1	T	0.3	2.1	1.4
R-6	T	ND	3.6	31.8
R-34e	I	29,450	326	NS ^f
Intermediate-Exposed				
R-7	I	ND	9.3	1.6
R-9	I	ND	NS	31.09
R-10	I	96	3.1	139
R-11	I	96	19	681
R-35	I	0.3	0.7	NS
R-36	I	ND	ND	1.8
R-37	I	ND	ND	0.6
R-38	P	ND	NS	0.7
Control				
R-19	I	ND	11.2	ND
R-20 ^d	P	1.1	13.5	63.1
R-23	P	0.8	1.2	ND
R-39	P	ND	1.3	0.8
R-40	I	ND	ND	0.6
R-41 ^d	P	0.7	1.4	ND
		Tetrachloroethylene		
Exposed				
R-1	T	0.8	1.4	3.3
R-6	T	ND	1.2	8.0
R-34	I	9.0	1.2	NS
Intermediate-Exposed				
R-7	I	ND	2.0	1.7
R-9	I	ND	NS	14.39
R-10	I	6.4	3.7	1.0
R-11	I	2.5	7.2	1.1
R-35	I	0.2	2.9	NS
R-36	I	ND	ND	2.7
R-37	I	ND	1.9	2.6
R-38	P	ND	NS	3.0

(continued)

TABLE 26 (continued)

Group and sample	Type of Water Supply ^b	Water Concentration $\mu\text{g/L}$	Air Concentration $\mu\text{g/m}^3$ ^c	
			Outside	Inside
Control				
R-19	I	ND	3.0	0.7
R-20 ^e	P	1.2	11.8	17.0
R-23	P	0.4	1.4	40.8
R-39	P	ND	1.6	2.0
R-40	I	ND	2.1	0.8
R-41 ^e	P	1.4	1.1	24.5

^aAll samples were analyzed for: hexachlorocyclopentadiene (HEX), hexachlorobicycloheptadiene (HEX-BCH), heptachloronorborene, chlordene, heptachlor, carbon tetrachloride and tetrachloroethylene.

^bI - Temporary supply; I - Individual wells; and P - Public supply.

^cInside samples were collected in a bathroom with water running. Outside samples were obtained within 10 meters of the house.

^dThis sample was obtained from a well recognized to be contaminated and thus no longer in use. The sample was also found to contain 2.5 $\mu\text{g/L}$ HEX-BCH and 390 mg/L chloroform. The air sample, collected during and after water was drawn from the well, showed 0.6 $\mu\text{g/m}^3$ HEX-BCH.

^eThe indoor air samples had 4.6 and 1.5 $\mu\text{g/m}^3$ of heptachlor for R-20 and R-41, respectively. The source is unknown.

^fNS - Indicates that no sample was obtained.

^gAn air sample from a second bathroom in this home contained only 0.8 $\mu\text{g/m}^3$ of carbon tetrachloride and 0.6 $\mu\text{g/m}^3$ of tetrachloroethylene.

No carbon tetrachloride was detected in any individual wells that were not on the Toone-Teague Road.

A summary of the air and water sampling results for January and March 1979 combined are presented in Table 27. Carbon tetrachloride and tetrachloroethylene were detected in most air samples, regardless of the group tested. Individual private well samples, except those along Toone-Teague Road did not contain detectable amounts of either compound.

DISCUSSION AND CONCLUSIONS

The concentrations of the suspected carcinogen carbon tetrachloride found in the contaminated well water (up to about 30,000 ppb) are far above the level of 2.6 ppb that has been proposed as a safe level for water (42). A survey of 80 municipal water supplies conducted by the U.S. Environmental Protection Agency in 1975 revealed that about 10% of the supplies had detectable levels of CCl_4 ranging from 2 to 3 $\mu\text{g/L}$ (ppb) (40). Another study of 113 public water supplies reported in 1977 confirmed the earlier survey (10% positives range; 2.4-6.4 $\mu\text{g/L}$) (41). Concentrations of carbon tetrachloride in the public water supplies used by some of the control group in this study ranged up to 1.0 ppb. The releases of CCl_4 into the Ohio River in February 1977 resulted in surface water concentrations as high as 340 $\mu\text{g/L}$ (ppb) (42,43). Concentrations in the contaminated wells have frequently been of the order of 5,000 $\mu\text{g/L}$. The normal background atmospheric concentrations of CCl_4 found in the continental air mass ranges from 0.78 to 9.1 $\mu\text{g/m}^3$ (ng/l) (42,44). Although some urban areas experience higher levels; for example, in Bayonne, N.J. levels as high as 117 $\mu\text{g/m}^3$ have been found (45). The highest level observed in an outside sample in this study was 326 $\mu\text{g/m}^3$.

Carbon tetrachloride is regarded as a highly toxic substance (46). It is absorbed through the lungs, gastrointestinal tract, and the skin. Although gastrointestinal and hepatorenal injuries and nervous system symptoms result from both ingestion and inhalation, gastrointestinal and hepatorenal injuries are most prominent in poisoning from ingestion, and nervous symptoms predominate in inhalation poisoning. In all poisonings the liver is the most drastically affected organ but kidney injury may be more prominent clinically. The lowest concentration in air that has produced any toxic effect in man is 130 mg/m^3 .

Lauria and Bogden (47) have recently reviewed the adverse effects from limited exposures to CCl_4 . They concluded that exposure to low levels of CCl_4 in air (63-157 mg/m^3) is not toxic but that at greater exposure concentrations, nausea, anorexia, headache, dizziness, lassitude, and vomiting were observed. Liver function abnormalities (elevation of transaminase and LDH) were also noted. The symptoms disappeared promptly when removed from the source of exposure. At increased exposures, nausea and vomiting were more prominent, accompanied by severe abdominal pain. It is also believed that any exposure to air concentrations above 628 mg/m^3 can be associated with severe liver and/or kidney damage, and that far less severe disease may be induced by exposures over 157 mg/m^3 . The review also showed that acute liver damage can be induced readily in a variety of animal species. The

TABLE 27. COMPARISON OF WATER AND AIR SAMPLES OF RESIDENCES
OF STUDY PARTICIPANTS (JANUARY AND MARCH 1979)

Group and sample ^b	Carbon Tetrachloride ^a			Tetrachloroethylene ^a		
	Water	Air Samples		Water	Air Samples	
	N _p /N _T	Outside	Inside	N _p /N _i	Outside	Inside
	(Range) ^c	N _p /N _T (Range)	N _p /N _T (Range)	(Range)	N _p /N _T (Range)	N _p /N _T (Range)
Exposed						
T	1/4 (0.3)	4/4 ^d (0.5-75)	4/4 (0.6-31.8)	1/4 (0.8)	3/4 (1.2-1.4)	4/4 (3.3-9.0)
I	1/1 (29,450)	1/1 (326)	NS ^e NS	1/1 (9.0)	1/1 (1.2)	NS NS
Intermediate-Exposed						
I	5/14 (0.3-96)	5/7 (0.4-19)	8/8 (0.6-681)	3/14 (0.2-6.4)	5/7 (0.3-7.2)	8/8 (0.6-14.3)
P	0/1	NS NS	1/1 (0.7)	0/1	NS NS	1/1 (3.0)
Control						
I	0/18	4/5 (0.2-11.2)	3/5 (0.2-0.6)	0/18	5/5 (0.3-3.0)	4/5 (0.3-0.8)
P	3/8 (0.7-1.1)	7/7 (0.1-13.5)	4/7 (0.2-63.1)	3/8 (0.4-1.4)	7/7 (0.2-11.8)	7/7 (0.2-40.8)

^aWater concentrations are ng/L; air concentrations are $\mu\text{g}/\text{m}^3$.

^bT designates temporary water supply; I - individual well; P - public supply.

^cN_T designates total number of samples collected; N_p designates the number with detectable levels.

^dOne air sample also showed 3.2 $\mu\text{g}/\text{m}^3$ of HEX-BCH.

^eNS indicates no sample.

liver damage is found to be ordinarily repaired if the insult is limited in dosage, and the repair may be complete even if necrosis and fibrosis have occurred. From animal feeding studies, 10 mg/kg body weight per day was suggested as safe for the rat and a dosage of 10 ppm in the human diet is considered a safe dose providing a greater than 100-fold safety factor, based on a calculated dosage of 0.07 mg/kg body weight. Existing evidence from human experience did not indicate that exposure to small quantities of CCl_4 in amounts below the threshold producing hepatic necrosis had any capability to produce severe damage to either liver or kidney. Human experience did not indicate that CCl_4 is a direct carcinogen. A committee of the National Academy of Sciences/National Research Council has suggested that at a dose of $1 \mu\text{g}/\text{day}$ there is a 5×10^{-8} lifetime risk of cancer (46). The U.S. Environmental Protection Agency has proposed a level of $0.26 \mu\text{g}/\text{L}$ in ambient water that would limit the risk to one case of cancer in one million population (42). Assuming a consumption of one liter per day, the individuals using the contaminated water had a dose of carbon tetrachloride of about 5 mg/day which corresponds to 0.083 mg/day/kg body weight for a 60 kg individual.

Among the most toxic chemicals known to have been dumped at the Hardeman County site are hexachlorocyclopentadiene (HEX) and hexachlorobicycloheptadiene (HEX-BCH). The occupational air standard (36) for HEX is $0.1 \text{ mg}/\text{m}^3$, much lower than that for carbon tetrachloride ($65 \text{ mg}/\text{m}^3$) which reflects that it is a much more toxic compound. HEX is a semi-volatile, highly reactive chlorinated compound which is only slightly soluble in water. Information concerning human health effects of exposure to HEX is very limited. What little is known about acute human toxicity is based largely upon isolated spills or other accidents involving pesticide workers, laboratory workers or others having occupational contact with HEX (48-50). Inhalation, dermal and oral exposures of HEX have been reported to cause several short term toxicological symptoms (51). HEX is a potent irritant and is readily absorbed through the skin. Industrial workers have experienced irritation of the eye and upper respiratory tract, and headaches upon exposure to HEX vapors; and burns upon contact with the liquid. Animal studies have indicated that long term exposures may result in a number of nonspecific adverse health effects (51). There is no information available regarding the health effects of chronic exposure to HEX. There have been no reports of epidemiologic studies of workers who are occupationally exposed to HEX (52-53). There is no information available concerning the potential health effects resulting from chronic environmental exposure to HEX via contaminated air and/or water. Even less is known of the toxic effects of HEX-BCH, but there are indications that HEX-BCH may be as toxic as HEX (54). If so, it may be estimated that the Threshold Limit Value (TLV) for HEX-BCH would be about equal to the TLV for HEX ($0.1 \text{ mg}/\text{m}^3$). In practice when evaluating air exposure levels for two or more hazardous substances, their effects should be considered to be additive unless other information is available. This practice implies that for complex exposure situations, i.e., those that might be encountered with contamination of a groundwater supply by a multi-component toxic dump leachate, determination of exposure levels of a single chemical may not

adequately reflect the actual exposure burden experienced by an individual. In this case, consideration of the exposure levels of several substances is recommended.

Exposure to many agents including drugs, alcohol, and a variety of chemicals as well as disease states including hepatitis can cause liver damage. However, based on the results of this study, which excluded many of these potential causes, the conclusion is reached that the most likely reason for the presence of liver abnormalities discovered in the inhabitants of the Toone-Teague Road area in November 1978 was the consumption of water from wells contaminated by the leaching of material from the chemical land dump located in that area. The acute symptomatology and the abnormal liver function tests have in all but a few cases returned to normal and with no further contaminations, should remain normal. However, it is impossible at this time to predict whether there will be any future chronic effects as a result of this exposure. Six of the seven individuals found to have enlarged livers were in the exposed group. A medical follow-up of the people studied in this investigation is warranted.

Preliminary toxicity and mutagenicity testing (55) has shown that contamination of some of the well water samples was sufficient to cause a toxic response and, in one, a slight mutagenic response. The well water samples were tested for this biological activity without any concentration step in the laboratory procedure. Municipal drinking water from Cincinnati or New Orleans requires concentration of the organic components to elicit a biological response. HEX-BCH, reported to cause a mutagenic response in the Ames assay (56), was detected in the contaminated well water as were the suspected carcinogens, chloroform and carbon tetrachloride.

The water sample collected from a heavily contaminated well in March 1979 contained the highest concentration of carbon tetrachloride found in that area up to that time. This result indicates that contamination of the aquifer is increasing even with decrease in the overall usage of the aquifer as a water supply. Future contamination of the much deeper artesian aquifer, which is used at a location about 50 miles away by Memphis, Tennessee, is a distinct possibility because abandoned well shafts to this aquifer could be plugged at the present time and, therefore, not be detectable by the static head tests performed by the U.S. Geological Survey (57).

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

FOR USE
ONLY
IF NEEDED

DESIGN
HEAD
KNOW
SECTION
HERE

SECTION 6

EVALUATION OF WORKER EXPOSURE TO PESTICIDE CHEMICALS AT A MEMPHIS, TENNESSEE, WASTEWATER TREATMENT PLANT

During a prospective study aimed at evaluating the health risks from bacteria and viruses associated with the treatment of municipal wastewater, an opportunity developed to investigate human exposures to toxic organic chemicals emitted from the wastewater being treated at one of the plants in this study. The particular plant in question was the North Wastewater Treatment Plant in Memphis, Tennessee.

This plant, which began operation during the summer of 1977, is located five miles from a manufacturer that produces and utilizes several chlorinated organic intermediates for the synthesis of flame retardants and pesticides (notably isodrin, endrin, chlordane and heptachlor). Waste from this manufacturer is discharged into a sewer that flows to the Memphis North Treatment Plant. Prior to construction of this treatment plant, wastewater from the areas served by this plant flowed directly to the Mississippi River or to one of its tributaries (60). Concern for potential exposure of treatment plant employees to toxic chlorinated organics prompted an investigation (61) during August and September 1977 to evaluate the potential hazards that might result from the initial diversion of industrial wastewater to the newly completed municipal wastewater treatment plant. The preliminary investigation revealed that air in the wet well of the treatment plant caused eye irritation and had a strong chemical odor similar to that at the pesticide manufacturing plant. Concentrations of hexachlorocyclopentadiene (HEX) in the air samples analyzed were below the detection limits of 0.008 $\mu\text{g}/\text{l}$ for the analytical procedures used. At the request of Region IV, U.S. Environmental Protection Agency, the National Institute of Occupational Safety and Health (NIOSH) made an evaluation of the potential problems associated with allowing HEX to flow into the Memphis North Treatment Plant. Concern over exposing workers to this toxicant was expressed, and it was recommended that, "The workers should not deliberately be used as test subjects in evaluating the toxic hazard from HCCPD." (62) HCCPD is an alternative abbreviation to hexachlorocyclopentadiene.

In the early part of 1978, following the initial evaluation, workers at this same plant complained of acute symptoms of respiratory distress, dizziness, headache, and irritation of the eyes, throat, nose, lung and skin. It was again noted that the plant had an unusual odor characteristic of industrial chemical wastes. The incidence of these acute symptoms was reported to be associated with periods of more intense chemical odor. In March 1978, during an apparent slug release of the pesticide materials, six workers on the night shift became ill with symptoms of loss of equilibrium, severe

BEGIN
LAST LINE
OF TEXT

DO NOT WRITE IN
THIS SPACE
FOR USE
ONLY IF NEEDED

headaches, and nausea. Two were taken to a local emergency room, examined and released with no definitive diagnosis.

Although it has been generally recognized that workers in sewage treatment plants may be at an increased risk of exposure to infectious agents (1), only recently have studies revealed incidences of exposure of sewage treatment plant workers to potentially toxic chemicals (64-66). Workers may have direct contact with chemical waste materials during solids removal and other operations; however, the primary route of exposure of treatment plant workers to these substances is probably by inhalation. Chemical wastes enter the treatment plant either in a soluble form or adsorbed to suspended materials in the wastewater. Aeration during the treatment processes can cause aerial dispersion of these substances as aerosols and vapors.

The symptoms reported by the workers in the present study were similar to those experienced by workers approximately a year earlier at the Morris Forman Wastewater Treatment Plant in Louisville, Kentucky (64,65). In that incident, exposure was determined to come from pesticide intermediates, hexachlorocyclopentadiene (HEX) and octachlorocyclopentene (OCTA), which were illegally dumped into the municipal sewer system. Ambient air levels of HEX several days after the plant was closed ranged from about $3,000 \mu\text{g}/\text{m}^3$ to $10,000 \mu\text{g}/\text{m}^3$, which far exceeded the recommended maximum eight-hour time-weighted average exposure level of $100 \mu\text{g}/\text{m}^3$ (67). Because of the similarities in these events in Louisville and Memphis, an investigation to determine the degree of exposure to these chemical contaminants at the Memphis sewage treatment plant was undertaken.

STUDY DESIGN

In view of the intermittent occurrence of acute symptoms experienced by the workers, a study was undertaken during May to September 1978, to determine if evidence of exposure to the toxic chemicals and absorption of same could be detected by use of biological testing. For comparison purposes, a second plant in the Memphis area, the Maxson Wastewater Treatment Plant, was used. The sewer from the pesticide manufacturer was not connected to the Maxson Plant so it was felt that exposure there should be low to nonexistent from that route of entry. The location of these two wastewater treatment plants and the pesticide manufacturer are shown in Figure 3. Three studies were performed during the May-September 1978 period. The protocol for these involved four elements which are illustrated in Table 28 and are elaborated as follows: (1) illness symptom questionnaire, (2) analysis of urine specimens for selected organic compounds, (3) liver and bile acid determinations, and (4) air and wastewater monitoring. In addition, during July an opportunity arose to collect urine specimens from several groups of workers including some workers at the North Plant during a period of chemical odors.

The initial objective of this study was to establish a baseline chemical exposure, if any, of the wastewater treatment plant workers during normal operational conditions so that if a spill incident occurred or employees experienced acute symptoms an assessment of a potential chemical exposure could be more readily made. Urine samples were collected during the second

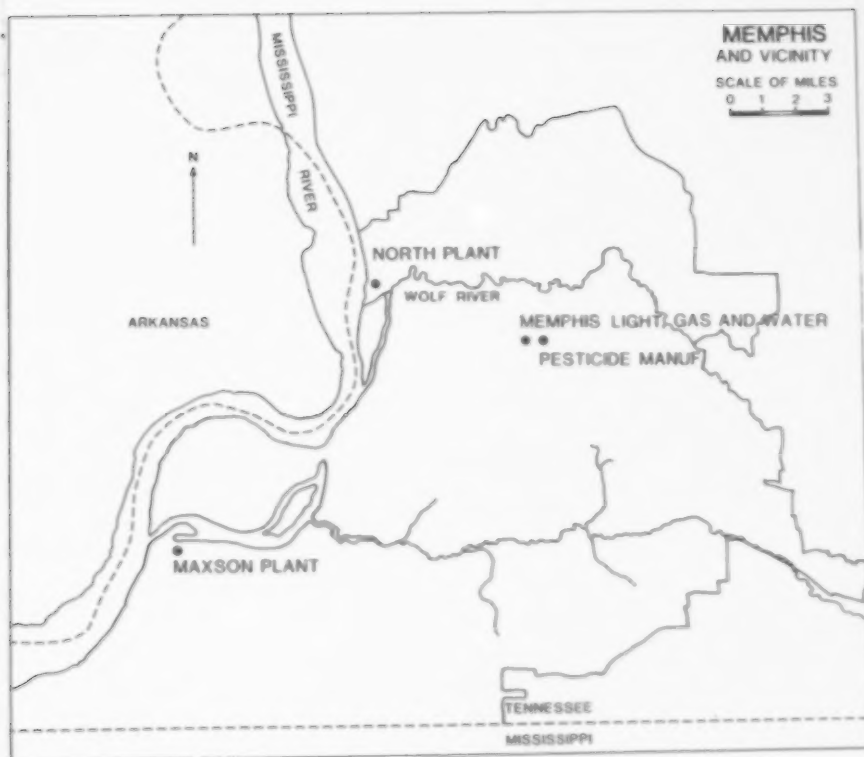


Figure 3. Location of Memphis Wastewater Treatment Plants, pesticide manufacturer and Memphis Light, Gas and Water.

TABLE 28. BASIC ELEMENTS OF EXPERIMENTAL DESIGN

Element	May 1978	Time of Study June 1978	September 1978
Illness symptom questionnaire	X	X	X
Urine analysis -			
early in shift		X	
late in shift	X	X	X
Liver and bile acid determinations			X
Wastewater monitoring	X	X	X
Air monitoring			
area	X	X	X
personal			X
Groups Tested			
Memphis North Plant	X	X	X
Maxson Plant	X	X	X
MLG&W Workers			X
Mill Creek Plant			X

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER IF
PAGE

TOP OF
PAGE
AREA

half of the work shift in May 1978 for this purpose. In addition, a limited environmental monitoring survey was performed to determine a) if the influent wastewater contained selected organic contaminants, and b) if so, were these substances being released into the air during the treatment processes.

CHOPPED
HEAD
BEGIN
SECTION
HERE

Follow-up urine screenings were conducted during June and September 1978. Urine samples at both the start and end of the work shift were collected in June 1978. During the September 1978 screening end of shift urine samples were also obtained from a group of workers at the Mill Creek Treatment Plant in Cincinnati, Ohio, and from workers at the Memphis Light, Gas and Water (MLG&W) Company. The MLG&W workers served as a control population for infectious disease studies of Memphis sewage treatment plant workers and thus, were included in this study as a population of workers with no association with the sewage treatment plants. The Cincinnati workers served as an additional control. In conjunction with the collection of urine specimens each employee was administered a questionnaire eliciting a number of symptoms and potential work-related problems. The interview related to the May urine collection was administered at the same time as that for June, requiring an approximately 30-day recall. Liver function tests and serum bile acid analyses were performed on blood specimens of participants in the ongoing seroepidemiological study to aid in a determination of possible chemical toxicity involving the liver.

During both June and September 1978 area air sampling was performed to provide an estimate of exposure levels of workers and measurements were made of the influent wastewater to determine the concentrations of selected organic contaminants present. Personal air monitoring of a group of workers at the Memphis North and Maxson wastewater treatment plants was conducted in September 1978. Eight-hour samples were collected at their work site and also while they were away from work.

Analytical Methods

The urine specimens were frozen immediately after collection and were kept frozen until analyzed. The frozen urine specimens were thawed in a cold water bath. A 5.0 mL aliquot of urine was placed in a screw cap tube, saturated with sodium chloride and mixed vigorously for 5 minutes with 5.0 mL of pesticide grade petroleum ether. The petroleum ether extract was dried over anhydrous sodium sulfate and stored in a freezer until analyzed. The extracts were analyzed for HEX and hexachlorobicycloheptadiene (HEX-BCH) by gas chromatography (GLC) using an electron capture detector. The analyses were performed with glass columns packed with OV-101 and OV-17/QF-1 at 175°C. Spehar et al employed a similar extraction and GLC analysis procedure for measuring the concentration of HEX, HEX-BCH and heptachlorobicycloheptene in water samples (67).

Air sampling consisted of drawing air through glass sampling tubes containing 150 mg of pre-extracted Chromosorb 102 (69,70). Desorption of the chlorinated organics was accomplished by transferring the sorbent to a vial, adding 2.0 mL of petroleum ether and allowing the mixture to stand for 1 hour with occasional shaking. A 5 μ L aliquot was subsequently analyzed by gas chromatography using an electron capture detector. This method is essentially

BEGIN
LAST LINE
OF TEXT

USE THESE
SPACE AREAS
FOR TABLES
AND FIGURES
IF NECESSARY

the same as the sampling and analysis procedure for HEX recommended by NIOSH (71) except that Chromosorb 102 was used as the sorbent rather than Porapak N. Commercially available Chromosorb 102 gave numerous peaks in the gas chromatograms under the desorption and analysis conditions. Removal of these impurities was achieved by successive extraction of the sorbent in a Soxhlet extractor with methanol, acetone, and petroleum ether. The sorbent was dried in a vacuum oven and pre-tested immediately before use.

The concentrations of the chlorinated organics in wastewater samples were determined by saturating an aliquot of the wastewater with sodium chloride, extracting with an equal volume of petroleum ether. A 5 μ L aliquot of the extract was injected into the gas chromatograph (72).

Statistical Methods

The distributions of the measurements of hexachlorocyclopentadiene and hexachlorobicycloheptadiene were skewed to the right. Following the use of the logarithmic (base 10) transformation, these variables now possessed unimodal, symmetric, bell-shaped distributions. The numbers of symptoms were transformed by taking their square roots. In order to evaluate the relationships among the above two variables, the symptoms of interest, and the covariables age, race, sex, date of measurement, work shift, time of measurement within work shift, distance from North Plant of residence, several analyses of covariance were performed. The dependent variables were the symptoms of interest and the above two variables. Each of these analyses of covariance were done in the following sequential manner due to the fact that several workers had one or more measurements of covariables missing from the data. Initially, all independent variables were included in the covariance model. Following this first analysis, those independent variables which were clearly nonsignificant ($p > 0.50$) were excluded from the model. Following the second analyses, those variables which were nonsignificant ($p > 0.25$), were excluded from the model. In the final analysis, those variables with $p < 0.05$ were taken to be significant.

RESULTS

Urine Analysis and Illness Symptoms

Age, race and sex characteristics of the participants in the May and June 1978 screenings are presented in Tables 29 and 30 and those for the September 1978 screening in Tables 31 and 32.

An examination of the results of the urine analyses for the May collections, during the second half of the work shift only, revealed that concentrations of HEX-BCH in specimens from North Plant workers tended to be higher than those from Maxson Plant workers (Table 33). No differences were detected in the HEX concentrations (Table 34). HEX-BCH was present in 84% of the North Plant samples and in only 34% of those from the Maxson Plant. Sixty-nine percent of North Plant samples contained greater than 3 μ g/l of HEX-BCH, while the percent of South Plant samples in this concentration range was 10% ($p < 0.001$). Concentrations of HEX in urine were not statistically different between the two plants.

TABLE 29. AGE DISTRIBUTION OF PARTICIPANTS IN MAY AND JUNE 1978
URINE SCREENINGS

	Age Range, Years				
	15-24	25-34	35-44	45-54	55-64
<u>May 1978</u>					
North Plant*	9	28	2	1	1
Maxson Plant	4	19	3	3	0
Total	13	47	5	4	1
<u>June 1978</u>					
North Plant**	20	27	6	4	1
Maxson Plant	3	12	4	2	3
Total	23	39	10	6	4
<u>Number of individuals tested at least once</u>					
North Plant	23	29	7	4	1
Maxson Plant	4	23	6	3	3
Total	27	52	13	7	4

*Age not available for one person.

**Age not available for two persons.

Number of Individuals
tested one or both dates75

TABLE 31. AGE DISTRIBUTION OF PARTICIPANTS IN SEPTEMBER 1978
URINE SCREENING

	Age Range, Years					Total
	15-24	25-34	35-44	45-54	55-64	
North Plant	14	26	5	3	5	53
Maxson Plant	7	16	2	2	2	30*
MLG&W	2	11	3	3	6	25
Mill Creek Plant**	5	12	2	1	0	20
All Locations	28	65	12	9	13	128

*Age of one participant unknown.

**Screening occurred in October 1978.

TABLE 32. RACE AND SEX DISTRIBUTION OF PARTICIPANTS IN
SEPTEMBER 1978 URINE SCREENING

		Male	Female	Total
North Plant	White	41	2	43
	Black	7	3	10
	Total	48	5	53
Maxson Plant	White	17	1	18
	Black	10	2	12
	Total	27	3	30
MLG&W	White	15	0	15
	Black	9	1	10
	Total	24	1	25
Mill Creek Plant*	White	15	0	15
	Black	4	0	4
	Total	20**	0	20
All Locations	White	88	3	91
	Black	30	6	36
	Total	119**	9	128

*Screening occurred in October 1978.

**Race of one participant not known.

TABLE 33. HEXACHLOROBICYCLOHEPTADIENE (HEX-BCH) CONCENTRATIONS IN URINE
SAMPLES OF MEMPHIS WASTEWATER TREATMENT PLANT EMPLOYEES
SECOND HALF OF WORK SHIFT, MAY 1978

Plant	Total Samples	Range $\mu\text{g/L}$	Urine Concentrations, $\mu\text{g/L}$		
			Below Detection	Less than or equal to 3	greater than 3
North ^a	32	< 0.3-8.4	5	5	22
South ^a	29	< 0.3-7.5	19	7	3

^a North Plant results higher than South Plant results ($p < .001$).

TABLE 34. HEXACHLOROCYCLOPENTADIENE (HEX) CONCENTRATIONS IN URINE
SAMPLES OF MEMPHIS WASTEWATER TREATMENT PLANT EMPLOYEES
SECOND HALF OF WORK SHIFT, MAY 1978

Plant ^b	NP/NT ^a	Range $\mu\text{g/L}$
North	5/32	< 0.8 - 2.0
South	2/29	< 0.8 - 1.4

^a NP = Number of samples containing the compound; NT = Total number of samples.

^b No significant difference between results from the two plants.

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD,
BEGIN
SECTIONS
HERE

The results of the analysis of urine specimens collected in June 1978 divided according to whether collected during the first half of the work shift or second half of the work shift are presented in Table 35 for HEX-BCH and Table 36 for HEX. A number of the urine samples obtained early in the work shift had detectable amounts of both HEX-BCH and HEX. In the case of North Plant samples some exposure could have occurred prior to obtaining the specimens. The HEX-BCH concentrations for the South Plant samples had one value of 12 $\mu\text{g/L}$ which was higher than the other samples. The values for the other nine positive samples ranged from 0.4 to 3.5 $\mu\text{g/L}$, while the values for the North Plant sample ranged from 0.4 to 7.5 $\mu\text{g/L}$. End of the work shift samples (Table 35) indicated that urinary excretion of HEX-BCH increased during the work shift for individuals at the North Plant ($p < 0.0001$). HEX-BCH excretion decreased during the work shift at the Maxson Plant and HEX excretion did not change significantly at either plant. Comparison of the results collected during the second half of the work shift in the May and June screening indicated that at the North Plant excretions of HEX-BCH were higher in May than in June ($p = < 0.010$) and excretions of HEX were not significantly different ($p = 0.051$). Thirty-three percent of the early in the shift samples had detectable concentrations of HEX-BCH. This increased to 76 percent for the end of shift samples and the mean for these was 4.1 $\mu\text{g/L}$.

The prevalence of self-reported illness symptoms during the May and June surveys, by plant and by shift, are presented in Table 37. The two symptoms of eye irritation or burning watery eyes; and fatigue or malaise were positively related to concentrations of HEX-BCH ($p = 0.002$ and 0.01 , respectively). For the May and June surveys there were also a number of significant statistical relationships among prevalence of various symptoms and plant, shift worked, age, sex, race, date of specimen, distance from North Plant and plant-shift interaction. The average number of symptoms per worker was 1.26 at the North Plant and 0.10 at the Maxson Plant ($p = 0.003$). The individual symptoms: headache, dizziness/ light headedness, vomiting/dry heaves and sleeplessness were more frequently reported in North Plant interviews. Interviews conducted during the day shift revealed an average of 0.43 symptoms; afternoon shift interviews, 1.50, and night shift interviews, 3.30 ($p = 0.0007$). The average number of symptoms per worker, by shift and by plant, are shown in Table 38. The plant-shift interaction symptoms was significant ($p = 0.04$). A plant-shift interaction was also found for the individual symptoms headaches ($p = 0.3$), dizziness and light headness ($p = .0008$) and tingling/ numbness of mouth and throat ($p = 0.0001$). These symptoms were reported infrequently or not at all at the Maxson Plant. While at the North Plant they were reported more frequently during the afternoon shift than during the day shift and for two of the symptoms they were reported most frequently during the night shift (Table 37). For 9 of the 16 symptoms there was a significant difference between shifts in the reported rates.

Presence of fatigue, malaise was positively correlated with age ($p = 0.003$). In sixteen percent of the interviews with male participants, nausea, lack of appetite, upset stomach or diarrhea were reported while none were reported in interviews with females ($p = 0.03$).

BEGIN
LAST LINE
OF TEXT

The average number of total symptoms per worker was lower for black workers 0.29, than for white workers, 1.07 ($p = 0.01$). There were fewer reports of the three individual symptoms of eye irritation, burning or watery

TOP OF
IMAGE AREA
OUTSIDE
EXTENSION
FOR TABLES
AND ILLUS-
TRATIONS

3/8"

78

PAGE NUMBER

TABLE 35. HEXACHLOROBICYCLOHEPTADIENE (HEX-BCH) CONCENTRATIONS IN URINE SAMPLES OF MEMPHIS WASTEWATER TREATMENT PLANT EMPLOYEES, JUNE 1978

Plant	Collection Time	Total Samples	Range $\mu\text{g/L}$	Urine Concentration, $\mu\text{g/L}$		
				Below Detection	less than or equal to 3	greater than 3
North	1st half shift ^a	54	<0.3-7.5	36	14	4
North	2nd half shift ^{a,b}	54	<0.3-10.3	13	19	22
Maxson	1st half shift	23	<0.3-12	14	7	2
Maxson	2nd half shift ^b	19	<0.3-1.6	16	3	0

a. 2nd half shift samples greater than 1st half samples ($p < 0.0001$).

b. 2nd half shift samples from North Plant greater than 2nd half samples from South Plant ($p < 0.0001$).

TABLE 36. HEXACHLOROCYCLOPENTADIENE (HEX) CONCENTRATIONS IN URINE SAMPLES OF MEMPHIS WASTEWATER TREATMENT PLANT EMPLOYEES, JUNE 1978

Plant	Collection Time	NP/NT ^a	Range $\mu\text{g/L}$
North ^b	1st half shift	11/54	<0.8-3.7
North ^b	2nd half shift	5/54	<0.8-3.9
Maxson ^b	1st half shift	5/23	<0.8-4.8
Maxson ^b	2nd half shift	5/19	<0.8-3.9

^aNP = Number of samples containing HEX; NT = Total number of samples.

^bThere were no statistically significant differences between plants or between 1st and 2nd half shift samples at either plant.

TABLE 37. PREVALENCE OF SELF-REPORTED SYMPTOM
DURING MAY AND JUNE SURVEYS

Symptoms	May						June						
	North Plant			Maxson Plant			North Plant			Maxson Plant			
	Shift			Shift			Shift			Shift			
	day	aft.	night	day	aft.	night	day	aft.	night	off	day	aft.	night
Number of interviews (number of workers)	36 (36)	3 (3)	3 (3)	23 (23)	5 (5)	1 (1)	87 (46)	10 (6)	12 (6)	3 (3)	42 (24)	0 (0)	0 (0)
1. Eye irritation, burning watery eyes	1 ^{a,d} (1)	1 ^{a,d} (1)	1 ^{a,d} (1)				10 ^{a,d} (6)	4 ^{a,d} (2)	2 ^{a,d} (1)	1 ^{a,d} (1)			
2. Fatigue, malaise					1 ^d (1)		8 ^d (4)						
3. Headache	a,b,c 1(1)	a,b,c 1(1)	a,b,c 2(1)				ab,c 8(4)	a,b,c 4(2)	a,b,c 8(4)	a,c,b 1(1)	a,b,c 2(1)		
4. Cough			1(1)				2(1)						
5. Chest discomfort	1 ^a (1)						2 ^a (1)		5 ^a (2)				
6. Dizziness, light- headedness			a,b,c 1(1)					a,b,c 3(2)	a,b,c 4(2)				
7. Wheezing, short- ness of breath difficulty in breathing			1 ^a (1)				1 ^a (1)	2 ^a (1)					
8. Fainting													
9. Nausea, lack of appetite upset stomach, diarrhea	a,d 1(1)		a,d 1(1)				a,d 10(5)	a,d 4(2)	a,d 9(5)	a,d 1(1)	a,d 4(2)		
10. Vomiting, dry heaves	a 1(1)	a 1(1)	a 1(1)				a 4(2)	a 2(1)	a 2(1)				
11. Irritation of upper respiratory, sore throat	a 1(1)	a 1(1)					a 4(2)		a 3(2)	a 1(1)			
12. Gagging			1(1)				2(1)						
13. Tingling/numbness of mouth & throat		a,d 1(1)						a,d 2(1)					
14. Loss of memory									1(1)	1(1)			
15. Irritability									1(1)	1(1)			
16. Sleeplessness			a,b 1(1)				a,b 2(1)		a,b 1(1)	a,b 1(1)			

a overall significant difference between shift
b overall significant difference between plant
c significant interaction between plant and shift
d significant difference between sampling dates

TABLE 38. AVERAGE NUMBER OF SYMPTOMS PER WORKER
PLANT AND SHIFT, MAY-JUNE 1978

	Shift			
	Day	Afternoon	Night	Off-Work
North Plant (number of workers)	0.63 (54)	2.43 (7)	3.44 (9)	2.33 (3)
Maxson Plant (number of workers)	0.09 (33)	0.20 (5)	0.00 (1)	-- (0)

eyes; fatigue, malaise; and tingling/numbness of the mouth and throat, from interviews with black workers than with white workers.

A higher rate of symptom prevalence was reported in June than in May ($p = 0.0053$). However, this may be due to the May survey of illnesses being conducted one month later. The prevalence difference also existed for the individual symptoms of eye irritation, burning watery eyes ($p = 0.04$), fatigue, malaise ($p = 0.008$), and nausea, lack of appetite, upset stomach, diarrhea ($p = 0.005$). There was a higher reported rate of the symptoms of headache, cough, vomiting and dry/heaves, gagging and sleeplessness for people living farther from the North Plant than among those living closer.

During July 1978 an additional 62 urine specimens were collected from several groups in Memphis, totalling 40 people. These groups consisted of North and Maxson plant employees, a crew of city employees sampling a sewer from the pesticide manufacturer and an additional control group not involved with either treatment plant. Results of analyses of these specimens are presented in Table 39. Six specimens were collected from four North Plant workers during a period of intense chemical odor. Two of the workers reported symptoms of dizziness, headache and drowsiness. Urine from one of these two workers contained $2.0 \mu\text{g/L}$ HEX-BCH three hours into the work shift and urine from the other contained $2.9 \mu\text{g/L}$ HEX at the end of the work shift. Only one other urine specimen of those evaluated in July 1978 contained a detectable amount of HEX. The sample was an end-of-shift specimen during the same period. HEX-BCH was detected in urine from four of the other 20 specimens collected from North Plant workers during July 1978 and in five of 20 specimens collected from the sewer sampling crew. One of the seven urine specimens collected from Maxson Plant workers and one of the nine from the other controls from elsewhere in Memphis contained detectable amounts of HEX-BCH. The positive sample from the Maxson Plant worker was collected at the start of the work shift, an end-of-shift sample from the worker did not contain detectable concentrations of HEX-BCH. The other control worker with HEX-BCH was the wife of an agricultural chemical company employee. Combining the results from the North Plant workers and the sampling crew and comparing them with the Maxson Plant workers and the other controls, the results from the former groups are significantly higher (Table 40).

The data presented in Table 41 gives the results of a urine screening conducted in September 1978 at the time of the regular quarterly specimen collection for the seroepidemiologic study. One individual at the North Plant had detectable concentration of urinary HEX-BCH. HEX was not found in any of the North Plant urine samples and neither HEX nor HEX-BCH was found in the samples of individuals from the Maxson Plant or from the Mill Creek Plant in Cincinnati, Ohio. However, HEX-BCH was detected in the range of $0.3\text{--}1.0 \mu\text{g/l}$ in the urine of seven of 26 MLG&W employees tested. An air sample at MLG&W contained 0.1 and $0.4 \mu\text{g/m}^3$ of HEX-BCH and heptachlorobicycloheptene, respectively. The prevalence of symptoms reported during the September 1978 interviews are shown in Table 42. The average number of symptoms reported per worker was North Plant, 0.29; Maxson Plant, 0.48; MLG&W, 0.16 and Mill Creek (Cincinnati), 0.07. The rate for the Maxson Plant increased from 0.08 to 0.48 from May-June to September while that for the North Plant decreased from 0.49 to 0.29.

TABLE 39. RESULTS OF ANALYSES URINE COLLECTED FROM VARIOUS GROUPS IN MEMPHIS, TENNESSEE DURING JULY 1978

Group	HEX		HEX-BCH	
	N _p /N _T ^a	Range ^b (μg/L)	N _p /N _T ^a	Range ^b (μg/L)
North Plant Workers ^c	2/6(2/4)	1.5-2.9	3/6(3/4)	2.0-2.9
North Plant Workers	0/20(0/12)	<0.8	4/20(4/12)	1.3-3.4
Sampling Crew	0/20(0/11)	<0.8	5/20(4/11)	1.1-4.0
Maxson Plant Workers	0/7(0/5)	<0.8	1/7 ^d (1/5)	1.3
Other Controls	0/9(0/8)	<0.8	1/9 ^e (1/8)	1.1

^aN_p = Number of samples containing the compound; N_T = total number of samples. Numbers in parenthesis refer to total number of workers positive/total number workers tested.

^bRange of samples with detectable amounts of compound.

^cCollected during period of intense chemical odors.

^dPerson with HEX-BCH was wife of agricultural chemical company employee.

^eSpecimen with 1.3 μg/L HEX-BCH was collected at start of shift, none was detected in end-of-shift sample.

TABLE 40. COMPARISON OF RESULTS OF HEX-BCH ANALYSES OF URINE SPECIMENS DURING JULY 1978 FROM NORTH PLANT WORKERS AND SAMPLING CREW WITH THOSE FROM MAXSON PLANT WORKERS AND OTHER CONTROL WORKERS

Worker Group	Total Workers	Range	Below Detection	Urine Concentration $\mu\text{g/L}$	
				Less than or Equal to 3	Greater than 3
Combined North plant and sampling crew	27	4.8-4.0	16	8	3
Maxson Plant and other controls	13	4.8-1.3	11	2	0

TABLE 41. URINE SAMPLES OF WASTEWATER TREATMENT PLANT EMPLOYEES COLLECTED AT THE END OF SHIFT IN SEPTEMBER 1978

Plant	HEX		HEX-BCH	
	N_p/N_T^a	Range ($\mu\text{g/L}$)	N_p/N	Range ($\mu\text{g/L}$)
North	0/55	<0.8	1/55	<0.3-0.8
Maxson	0/32	<0.8	0/32	<0.3
MLG&W	0/29	<0.8	7/29	<0.3-1.0
Mill Creek Cincinnati, Ohio ^b	0/20	<0.8	0/20	<0.3

^a N_p - Number of samples containing the compound; N_T - Total number of samples.

^b Collected in October 1978.

TABLE 42. PREVALENCE OF SELF-REPORTED SYMPTOMS
DURING SEPTEMBER 1978 SURVEY

Symptom	Plant			
	North	Maxson	MLG&W	Mill Creek
(Number of Workers)	45	31	34	15
1. Eye irritation, burn- ing watery eyes		2		
2. Fatigue, malaise		2	1	
3. Headache	1	4	2	
4. Cough				
5. Chest discomfort	1			
6. Dizziness, light- headedness	1	2		
7. Wheezing, short- ness of breath, difficulty in breathing		1		
8. Fainting				
9. Nausea, lack of ap- petite, upset sto- mach, diarrhea	4	2		
10. Vomiting, dry heaves				
11. Irritation of upper respiratory system, sore throat	4	2	1	1
12. Gagging				
13. Tingling/numbness of mouth & throat				
14. Loss of memory				
15. Irritability				
16. Sleeplessness				
17. Other	2		1	
TOTAL	13	15	5	1
AVERAGE RATE	0.29	0.48	0.16	0.07

Summary of Results of Analysis of Urine Data and Illness Symptoms

Urine concentrations of HEX-BCH were significantly higher in North Plant workers than in Maxson Plant workers in both May and June 1978. The collection of specimens early and late in the work shift during June revealed an increase in urinary excretion during the work shift among North Plant workers and a decrease among those at the Maxson Plant. No differences between plants were detected for HEX. Between the May-June collections and the September collection there was an overall decline in urine concentrations of HEX-BCH and HEX. It should be noted that worker awareness of the presence of these compounds increased considerably after the June collections as the result of meetings and other communications and they were advised to leave work areas when the chemical odors were intense.

Workers at the North Plant reported a higher rate of illness symptoms during May and June than did Maxson Plant workers. The presence of eye irritation or burning, watery eyes and fatigue/malaise were positively related to urine concentrations of HEX-BCH. If the statistically significant increased prevalences of some of the symptoms detected are not due to chance alone, then they suggest that when far enough away from air contamination while not at work, they become more susceptible to them when at work. Because the urine concentrations of HEX and HEX-BCH were below detectable limits for all and almost all workers, respectively, in September, it is not possible to relate illness symptoms to urine concentrations for all groups tested in September.

Environmental Monitoring

Figure 4 shows typical gas chromatograms of influent wastewater collected at the North and Maxson treatment plants during the time of this study. The North Plant sample clearly shows the presence of several contaminants including HEX-BCH, heptachlorobicycloheptene and chlordene. In comparison, the influent samples at the Maxson Plant do not contain these types of contaminants. Although the presence of several contaminants in the wastewater of the Maxson Plant is indicated, they would have to be emitted into the air as aerosols or vapors before significant exposure to workers would occur.

Gas chromatograms of air samples collected at the wet well and grit chamber are similar in appearance to the chromatograms of the wastewater. HEX, HEX-BCH, heptachlorobicycloheptene and chlordene were present in the air samples at the North Plant. This suggests that airborne levels of these substances result from aeration stripping of the wastewater. Air samples have also been obtained at the grit chamber of the Maxson Plant and the Mill Creek Treatment Plant in Cincinnati, Ohio. Chromatograms of these samples revealed the presence of several early eluting components; however, there was no indication of the type of substances found in the samples obtained at the Memphis North Plant. Some of these early eluting substances were tentatively identified by gas chromatographic retention times as volatile chlorinated hydrocarbons including chloroform, carbon tetrachloride, and tetrachloroethylene.

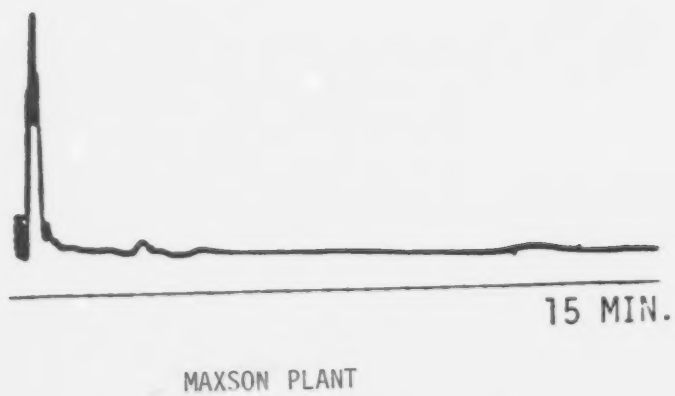
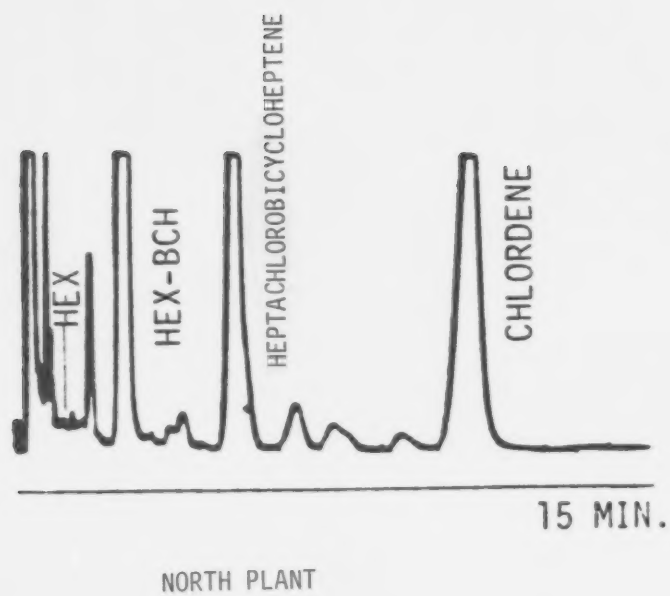


Figure 4. Gas chromatograms of extracts of influent wastewater samples.

The concentrations of four chlorinated organics found in the influent wastewater of the Memphis North Plant during the time of air sampling and urine screening studies are given in Table 43. Although these values do not represent the values for the complete month, they are indicative of the concentrations at various times. The concentration of HEX-BCH was generally about 300 ppb, although much lower levels were measured during October and November. Heptachlorobicycloheptene and chlordene concentrations seem to be more variable ranging from about 17 to 1980 ppb. One sample collected during September had a chlordene concentration of 1980 ppb. The September samples listed in Table 43 were collected at the time of a reported "spill" which occurred after the urine screening that month. The analysis of wastewater and air samples indicated that heptachlorobicycloheptene was at least one of the major constituents in the "spill."

Area air samples collected at various locations in the treatment plant provided information about the magnitude of aerial dispersion of these semi-volatile compounds. The concentrations of these substances found in air samples collected in the wet well, a building with a ventilation system, and the grit chamber, are presented in Table 44. The levels at the wet well were generally higher than at the grit chamber. HEX was found at levels as high as $39 \mu\text{g}/\text{m}^3$. The highest levels of HEX-BCH and heptachlorobicycloheptene were $280 \mu\text{g}/\text{m}^3$ and $200 \mu\text{g}/\text{m}^3$, respectively.

Personal air monitoring of 11 workers at the North Plant and 9 at the Maxson Plant was carried out in September 1978. Eight-hour personal air samples were collected during the work shift and during the workers' off-shift time (home sample). The work shift sample for most of the North Plant workers contained HEX, HEX-BCH, heptachlorobicycloheptene and chlordene (Table 45). There was no obvious relationship between work area and the extent of exposure. However, for North Plant workers the air concentrations for the four compounds found from personal air monitoring during the work shift were in good agreement with the concentrations determined from area air samples collected at the grit chamber. Several of the home samples of North Plant workers also had detectable amounts of some of these compounds, but the levels were considerably lower than the work shift sample. The samples of the workers at the Maxson (South) Plant were below detection limits except for one work shift sample which contained a trace of HEX-BCH. All urine samples contained less than detectable concentration of HEX, HEX-BCH and heptachlorobicycloheptene.

Medical

Liver function tests were performed in November 1977 and in November 1978. No differences between values for workers at the North Plant, Maxson Plant and MLG&W were detected for either year. Total serum bile acid determinations performed on the November 1978 serum did not reveal any significant differences between the groups.

DISCUSSION

Hexachlorocyclopentadiene (HEX) is a semi-volatile, highly reactive chlorinated compound which is only slightly soluble in water. Information

TABLE 43. CONCENTRATIONS OF SELECTED ORGANIC COMPOUNDS IN
INFLUENT WASTEWATER AT MEMPHIS NORTH TREATMENT PLANT, 1978

Date	Concentration, $\mu\text{g/L}$			
	HEX	HEX-BCH	HEX-VCL*	Chlordene
June	3	334	57	87
August	< 0.8	329	115	216
September	4	292	668	58
October-November	< 0.8	11	17	32

*Heptachlorobicycloheptene

TABLE 44. AREA AIR SAMPLES COLLECTED AT THE MEMPHIS
NORTH PLANT, 1978

A. WET WELL

Date	Concentration, $\mu\text{g}/\text{m}^3$			
	HEX*	HEX-BCH	HEX-VCE**	Chlordene
May	<0.03	219	87	45
June	18	278	15	16
September	8	25	200	44
October	15	2	1	0.1
November	39	7	85	7.8

B. GRIT CHAMBER

Date	Concentration, $\mu\text{g}/\text{m}^3$			
	HEX	HEX-BCH	HEX-VCL	Chlordene
May	<0.03	4.1	1.9	0.9
June	6.3	65	1.5	5.3
July	<0.03	0.5	0.7	2.3
September	<0.03	0.5	1.1	2.7
October	0.04	1.2	1.0	0.8
November	12	2.6	4.3	1.0

*Threshold Limit Value for eight hour sample is $110 \mu\text{g}/\text{m}^3$ (10 ppb).

**Heptachlorobicycloheptene

TABLE 45. PERSONAL AIR MONITORING OF WORKERS AT THE
MEMPHIS NORTH WASTEWATER TREATMENT PLANT

Worker	Concentration, $\mu\text{g}/\text{m}^3$							
	HEX		HEX-BCH		HEX-VCL ^d		Chlordene	
	W ^a	H ^a	W	H	W	H	W	H
1	0.1	-- ^b	0.12	0.02	0.09	0.04	0.20	--
2	--	NS ^c	0.02	NS	--	NS	--	NS
3	0.31	--	0.40	--	0.16	--	0.21	--
4	--	NS	0.46	NS	0.31	NS	1.25	NS
5	--	--	0.05	--	0.02	--	--	--
6	--	NS	0.22	NS	0.10	NS	--	NS
7	0.42	--	0.33	0.03	0.21	0.10	3.32	--
8	--	NS	0.37	NS	0.21	NS	1.19	NS
9	--	NS	0.24	NS	--	NS	--	NS
10	--	NS	0.48	NS	0.22	NS	0.94	NS
11	--	0.08	0.10	--	0.02	--	--	--

^a W - designates the work shift sample, H - designates the home sample.

^b(--) indicates the sample was below detection limit.

^c NS indicates that no sample was obtained.

^d Heptachlorobicycloheptene.

concerning human health effects of exposure to HEX is very limited. What little is known about acute human toxicity is based largely upon isolated spills or other accidents involving pesticide workers, laboratory workers or others having occupational contact with HEX. Industrial workers have experienced irritation of the eyes and upper respiratory tract, and headaches upon exposure to HEX vapors; and burns upon contact with liquid (73-75).

Mehendale (76) investigated the absorption, metabolism and excretion of a single oral dose of radiolabelled HEX (^{14}C -HEX) in laboratory animals (male rats). Approximately 29% of total ^{14}C dose was eliminated in the urine during the first 24 hours after administration. Other routes of elimination were feces and probably the respiratory tract. The metabolic pathway of HEX is uncertain, but the Mehendale study suggests the possibility of four metabolites. Several toxicological symptoms have been observed in mammals exposed to HEX by inhalation, dermal and oral routes of administration (73,74). Animal studies have also indicated that long term exposures may result in a number of nonspecific health effects.

In 1977 a Threshold Limit Value (TLV) of $100\text{ }\mu\text{g}/\text{m}^3$ was recommended by the American Conference of Governmental Industrial Hygienists (67). TLV's have not been established for the other compounds, but there are indication that HEX-BCH may be as toxic as HEX (68). Hypothetically, if it is assumed that the TLV for HEX-BCH would be equal to the TLV for HEX ($100\text{ }\mu\text{g}/\text{m}^3$ or 10 ppb), air concentrations measured in the wet well during May and June 1978 would be about two or more hazardous substances, their effects should be considered to be additive unless other information is available. This practice implies that for complex exposure situations, i.e., those that might be encountered at wastewater treatment facilities, determination of exposure levels of a single chemical may not adequately reflect the actual exposure burden experienced by an individual. In this case, consideration of the exposure levels of several substances is recommended.

In many industrial operations control measures can usually be instituted to minimize worker exposure and atmospheric release of toxic chemical contaminants. However, the many open tanks and basins in wastewater treatment facilities generally are not designed to prevent aerial dispersion of contaminants. Furthermore, the nature and quantity of chemicals in the influent wastewater are usually highly variable and generally unknown. In many systems aeration of the wastewater, such as in activated sludge treatment facilities, is a vital treatment process, but this also causes increased aerial dispersion of certain chemical contaminants as aerosols and vapors.

The source of the organic chemicals found in urine specimens and occasionally in air samples from the Maxson Plant is not well understood. During the June 1978 screening the percent of North Plant urine samples with HEX-BCH concentrations greater than $3\text{ }\mu\text{g}/\text{L}$ increased from 7 to 41 during the work shift while in Maxson Plant samples the percent decreased from 9 to 0. Possibilities for the presence of organic chemicals in urine of Maxson Plant workers include: intermittent discharge into the sewer system by either a user of these chemicals or a waste hauler, discharge from a buried dump site containing these substances, and ambient air pollution in the Memphis area.

GROUP
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
HERE

It is clear from the data collected during the study that these compounds are not present at the Maxson Plant at the same levels as at the North Plant. The small quantities of HEX-BCH detected in the urine of some MLG&W employees could have been due to the proximity of MLG&W to the pesticide manufacturer or to ambient levels at the sites of their work that day away from their central facility.

GROUP 11
FIRST
LINE OF
TEXT
HERE

At the present time there is inadequate information available regarding the health affects of chronic exposure to HEX and related chlorinated compounds. Epidemiologic studies of workers who are occupationally exposed to HEX are lacking and there is no information available concerning the potential health effects resulting from chronic environmental exposure to HEX via contaminated air and/or water. The Louisville, Kentucky episode (64,65) demonstrates a case of acute exposure to toxic contaminants from wastewater. The situation described in this report is a case of essentially continuous chronic exposures with intermittent acute exposures. Unfortunately, only limited information is available on the toxicity and health effects of these chlorinated organics. Thus, the significance of urinary excretion of these substances and the possible relationship to health effects is not known.

GROUP
LAST LINE
OF TEXT

GROUP 12
FIRST LINE
OF TEXT
HERE

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
PAGE
AREA

CHANGES
HERE
SHOW
SECTION
BEGINS

SECTION 7

VIRUSES IN AEROSOLS AND WASTEWATER AT A WASTEWATER SPRAY IRRIGATION SYSTEM

The purpose of this project was to collect, enumerate, and identify viruses in aerosols at a spray irrigation site, the Muskegon County, Michigan, Wastewater Management System Number 1, and to examine wastewater samples for the presence of coliphage and animal viruses in order to compare airborne levels to the levels in the wastewater. The presence of animal viruses in aerosols would indicate a potential health risk for treatment plant workers. Standard Plate Count bacterial analyses were also performed for comparison purposes. The sampler used in this study was the Army prototype XM2 Biological Sampler/Collector.

SITE DESCRIPTION

The Muskegon County Wastewater Management System Number 1 was a lagoon-impoundment, spray irrigation treatment facility which serviced 13 municipalities and five major industries (77,78). The system served a population of approximately 180,000. The average daily flow of wastewater was 106,000 M³ (28 million gallons). Approximately 50% of this flow came from a paper mill and another 5% from chemical manufacturers. The system consisted of a 4,450 hectare (11,000 acre) site which contained three aerated biological treatment cells (aeration basins), two storage lagoons of 345 hectares (850 acres) each with a total storage capacity of 19.3 million cubic meters (5.1 billion gallons); and 2,270 hectares (5500 acres) of land irrigated by 54 center-pivot irrigation rigs (Figure 5).

Raw waste entered aeration basins number 2 and 3 which were operated in series. Aeration basin #1 was not in use during this study. Each aeration basin had 3.2 hectares (8 acres) of surface area and was equipped with 12 mechanical surface aerators and 6 mixers. A settling pond and an outlet lagoon, located adjacent to the aeration basins, were also not in use during the study. After an average detention time of three days, the effluent flowed to the storage lagoons. The lagoons provided additional time for stabilization and settling of wastes and for storage of wastewater during the fall and winter seasons when irrigation was not practiced. The wastewater was not disinfected. Wastewater flowed from the lagoons via open channels to the two pump stations for distribution to the 54 irrigation rigs. These rigs, equipped with downward-directed aerated high pressure spray nozzles, have a radius of 229 to 396 meters (750 to 1300 feet) and a rotation rate that varied from 1 to 7 days. The wastewater application rate was approximately 5700 M³/day/rig during the growing season. An underdrainage

BEGIN
LAST LINE
OF TEXT

DO NOT
WRITE
OR
DRAW
ON
THIS
PAGE

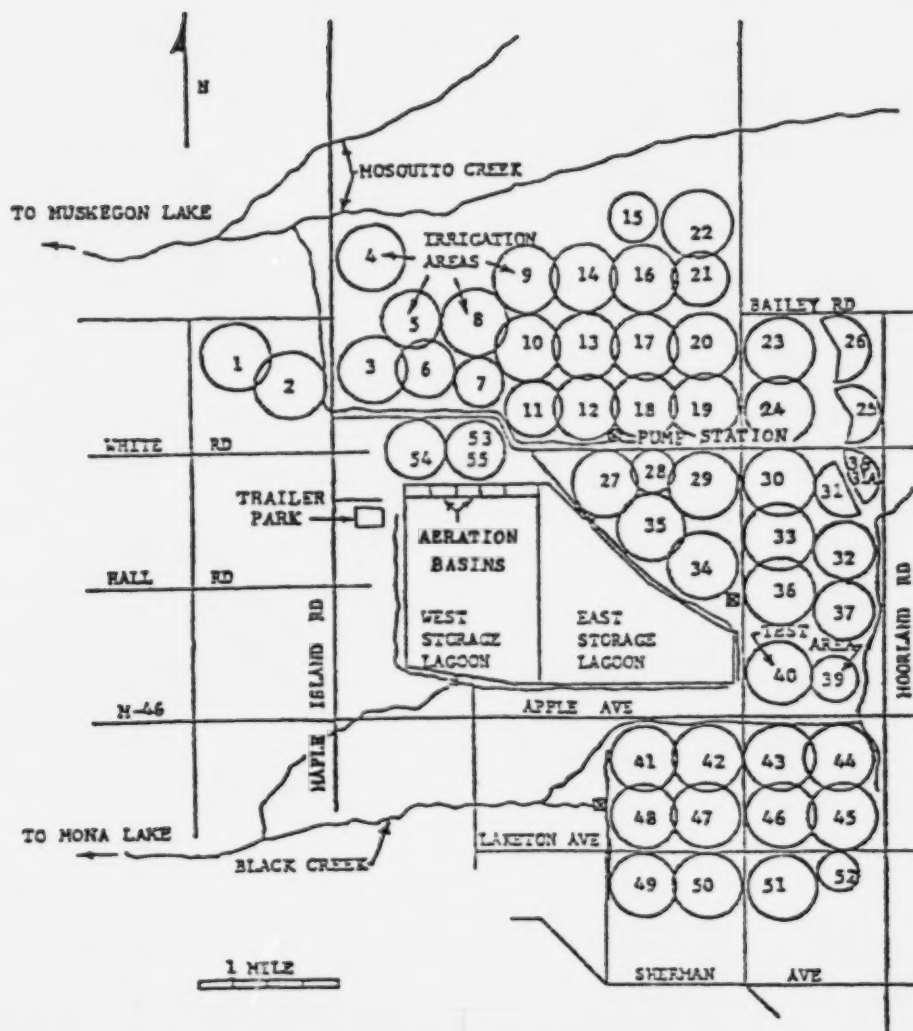


Figure 5. Muskegon County Wastewater Management System no. 1.

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

system collected leachate from the irrigated fields for discharge into nearby waterways.

METHODS

XM2 Biological Sampler/Collector

The Army prototype XM2 Biological Sampler/Collector, on loan from the Edgewood Arsenal, was manufactured by the Environmental Research Corporation. This sampler/collector removed airborne particles from the air flow, and concentrated (in an 8:1 ratio) classified particles (2-12 μm) in a smaller volume of air before moving them into a collection liquid via a combination of impingement and scrubbing. This apparatus could store discrete 10 ml portions of the spent collection liquid in a temperature-controlled environment or in a large reservoir, depending on which option was chosen. A schematic of the sampler air unit is shown in Figure 6. The sampler processed 1050 liters of air per minute and could run continuously for periods of time up to 24 hours without the addition of more collecting liquid (79). Sampling liquid was collected at a rate of approximately 2.0 ml per minute. A schematic of the sample gas flow through the XM2 Sampler/Collector is shown in Figure 7.

Collection Liquids

Sterile collection liquids used were Earle's Lactalbumin Hydrolysate (ELH) with antibiotics, ELH without antibiotics, and distilled water. Samples collected in ELH with antibiotics were assayed for viruses. Samples collected in ELH without antibiotics and distilled water were assayed for bacteria and coliphage.

ELH was made up using 100 ml 10x ELH stock (GIBCO), 870 ml sterile deionized distilled water, and 30 ml 7.5% NaHCO_3 stock. All stock solutions were sterilized by pressure filtration. When antibiotics were required, the following were added:

- 1.0 ml Penicillin-streptomycin stock, containing 100 Units/ml of Penicillin G and 100 $\mu\text{g}/\text{ml}$ Dihydro-streptomycin
- 0.2 ml Amphotericin B stock (1 $\mu\text{g}/\text{ml}$)
- 0.5 ml Tetracycline stock (12.5 $\mu\text{g}/\text{ml}$).

Measurement of the Air Velocity of the Sampler Air Unit

The air flow rate through the sampler air unit was checked using a probe-type air velocity meter (Model 441, Sierra Instruments, Inc.). A cardboard collar, 28" high and 4.8" in diameter was constructed and attached to the sampler air intake. Readings were taken by inserting the probe into an opening in the collar located one fifth the collar length above the air intake. The four readings, taken at different locations within the collar (see Figure 8), were averaged and multiplied by the cross-sectional area to obtain the air velocity. The sampler air velocity was within + 10-15% of the air velocity stated in the Operator's manual (1050 liters/minute) at the

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
DEFINE
DIMENSION
FOR TABLE
AND FIGURE
THAT HAVE

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
SECTION
HERE

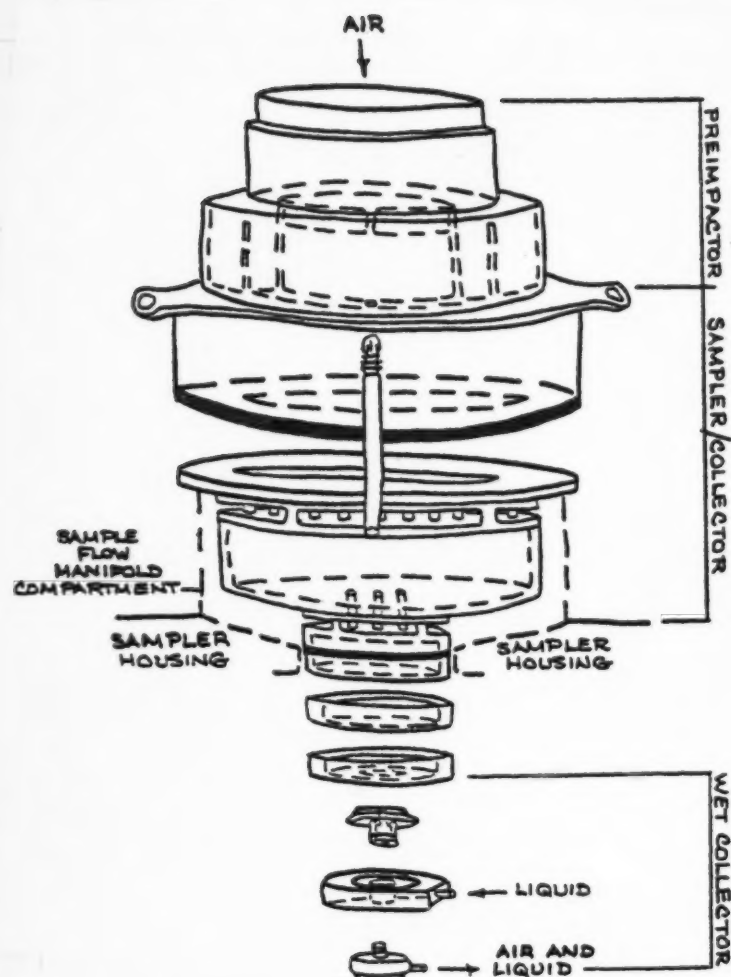


Figure 6. XM2 sampler air unit.

BEGIN
LAST LINE
OF TEXT

97

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

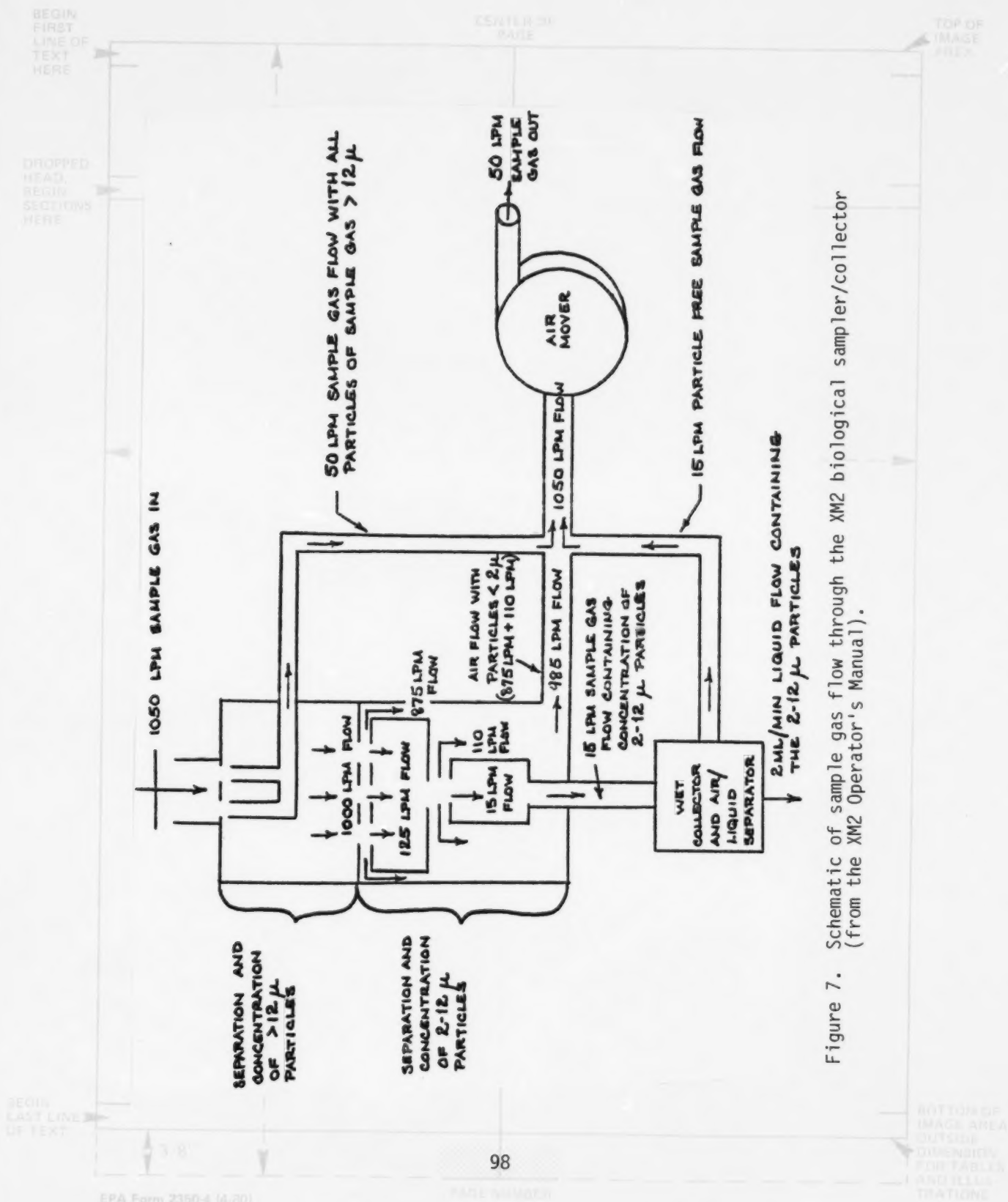


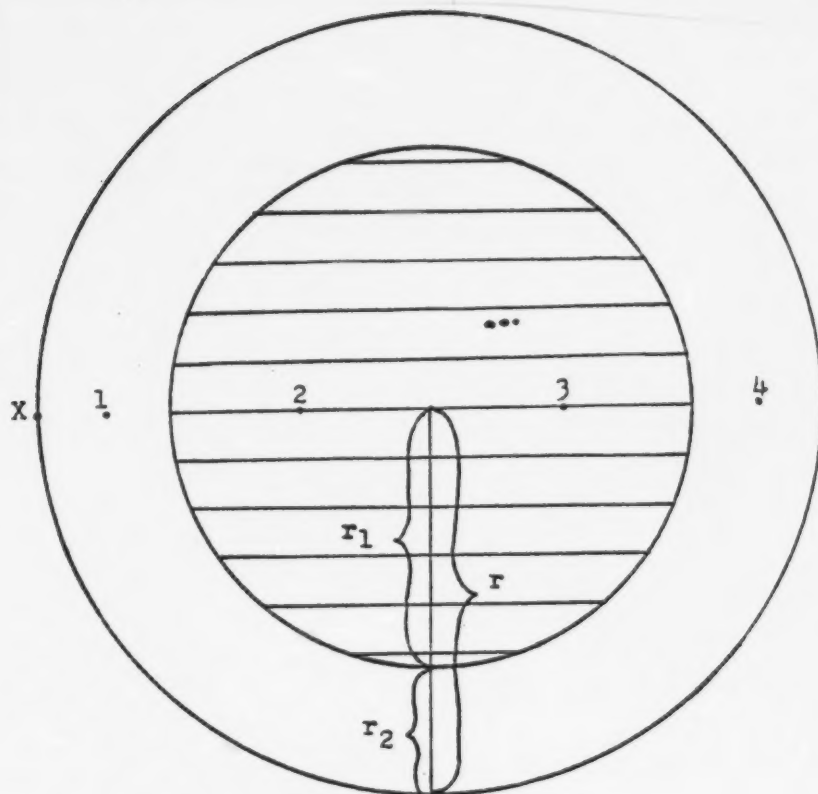
Figure 7. Schematic of sample gas flow through the XM2 biological sampler/collector (from the XM2 Operator's Manual).

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTION
HERE



$$\boxed{\text{Area}} = \boxed{\text{Area}}$$

Location Number	Distance From Probe Opening(X)
1	11/32"
2	1 9/16"
3	3 1/4"
4	4 7/16"

$$r = r_1 + r_2$$

$$r = 2.4" \text{ or } 0.2'$$

$$r_1 = 1.7"$$

$$r_2 = 0.7"$$

$$\text{Area} = \pi r^2$$

$$\text{Area} = \pi (0.2 \text{ ft.})^2$$

$$\text{Area} = 0.126 \text{ ft.}^2$$

Figure 8. Cross-section of the air collar of the sampler showing the location of the air flow readings.

BEGIN
LAST LINE
OF TEXT

1/8"

99

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND HEAVY
FRONT

settings recommended by the Edgewood Arsenal personnel. Variation in readings were due primarily to fluctuations in the blower motor speed.

Cleaning and Sterilization Procedures

The sampler tubing was sterilized by filling it with 100-200 mg/l Clorox solution, prepared in sterile distilled water, and allowing it to sit overnight. After flushing the tubing with freshly prepared Clorox solution, the tubing ends were immersed in sterile containers containing 100-200 mg/l Clorox solution. After a 1-hour contact time, the tubing ends were rinsed with sterile distilled water, and the tubing was flushed with sterile distilled water until a negative Cl_2 spot test using DPD #1 Powder (LaMotte Chemical Co.) was obtained. Aliquots of the upper and lower distilled water rinses were then collected in sterile containers to check for tubing sterility. These distilled water rinses were checked for the presence of bacteria, fungi, viruses, and coliphage. If any were found, the cleaning procedure was repeated. Sterile air was drawn into the lower tubing via a sterile trap system (see Figure 9). Sterile distilled water was left in the upper tubing until the sampling day. The ends of the tubing were given a final 1-hour contact time in Clorox solution, rinsed with sterile distilled water, and covered with sterile foil.

The sampler air unit was sterilized immediately after every run and again after cleaning. The air unit, wrapped in brown kraft paper, was exposed to ethylene oxide gas (12% by weight) for 4 hours in an Amsco Cryotherm, with 30-50% humidity. The air unit was then aerated for 2 hours in an Amsco Ethylene Oxide Gas Aerator. The air unit shroud was sterilized similarly. The wet collector of the air unit was cleaned after every sampling run, and the entire air unit was dismantled for cleaning every other run. Sterility of the air unit and shroud was checked by ethylene oxide sensitive tape and by the use of *Bacillus subtilis*, var. *niger* spore strips (Raven Biological Labs, Inc.), taped inside the air unit and shroud prior to sterilization. These were removed at the sampling site during sampler assembly, placed in the envelope with the positive control, and inoculated into thioglycollate broth upon our return to Cincinnati.

Sterility Tests

The distilled water rinses from the sampler tubing were checked for the presence of bacteria by inoculating several ml of each into Trypticase Soy Broth (TSB) tubes and incubating at 37°C for one week. The tubing was considered free of bacteria if there was no growth in TSB at the end of that time. At least 2 ml of each rinse were put on the surface of Trypticase Soy Agar (TSA) plates (1 ml/plate), and the inoculum was spread by sterile hockey sticks. The TSA plates were observed for fungal growth after room temperature incubation for 1 week. Aliquots of both distilled water rinses were assayed for viruses and coliphage by the usual procedure, described elsewhere.

The air unit, shroud, and control spore strip envelopes were cut with scissors that had been dipped in ethanol and flamed. The spore strips were poured aseptically into tubes of fluid thioglycollate that had been heated to drive off any absorbed oxygen and then cooled. The tubes of broth were

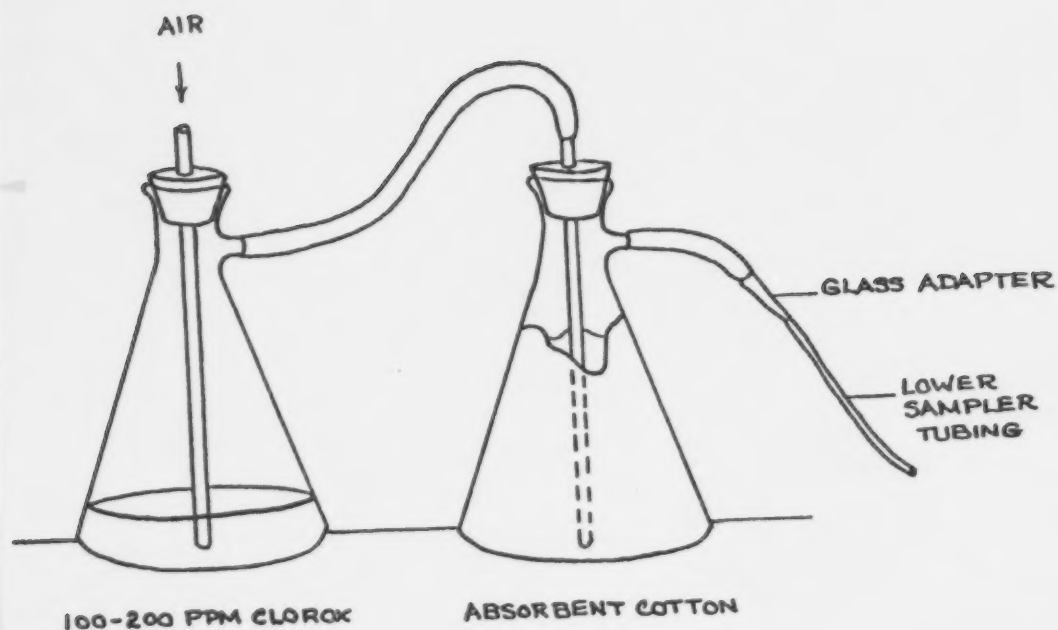


Figure 9. Sterile trap system for introducing air into lower tubing of the sampler.

incubated for one week at 37°C and checked for growth daily. Growth in the positive control tube and absence of growth in the other two indicated that the air unit and shroud were sterile. The air unit and shroud were found to be sterile in all runs.

Heathkit Portable Weather Station, Model ID-1290

This portable weather station was used to monitor weather parameters (temperature, relative humidity, barometric pressure, wind direction and velocity) during all air sampling runs. Readings were generally taken every 10-15 minutes. Cloud cover and other general weather conditions were also recorded.

Sampling Procedure

Samples were collected during six sampling trips from April to October, 1979. All aerosol samples were collected with the XM2 Biological Sampler/Collector at aeration basin #2, downwind of an operating surface aerator whenever possible. Figures 10 and 11 show the location of the sampling sites. These sites were upwind of the aeration basin when the wind was from the west or northwest.

The sampler was placed about 1 meter from the aeration basin wall (about 0.6 meters high) which was 2.5-3 meters from the edge of the basin wastewater. The wastewater surface was usually about 2 meters lower than the base of the wall. The sampler air intake was located above wall height, 0.7 meters from the ground or about 2.7 meters above the basin wastewater surface (see Figure 12). The sampler was located 20-25 meters from the center of the nearest operating surface aerator on all sampling dates, except 5/15/79, when it was located approximately 90 minutes down the road between aeration basin #1 and #2. In that position, it was 70-73 meters from the center of the nearest operating aerator.

After the weather station was set up at the sampling site the XM2 air unit was unwrapped and placed in the sampler. The sterile tubing ends were connected to the wet collector liquid inlet and outlet ports. Sampler hours were recorded, and the airflow settings were checked before the sterile collecting liquid was pumped into the tubing. The sampler case was then closed, and the shroud was put into place at the air inlet. Two 2-hour samples were collected; the first in ELH without antibiotics, and the second in ELH with antibiotics. On June 19, 1979, an additional sample was collected in distilled water prior to collecting the two samples in ELH. Weather parameters were monitored during all sampling runs.

As soon as possible after sampling, water samples were collected at the aeration basin manhole (raw influent), the west lagoon, and the ditch at the north pumping station, before and after the location where the liquid nitrogen was added to the wastewater. In addition, foam that accumulated on the surface of the aeration basin was repeatedly scooped into a sterile beaker and allowed to settle. When a sufficient quantity of settled foam was obtained, a small quantity of ELH without antibiotics, distilled water, or 10% beef extract, pH 7.0, prepared in McIlvaine's citric acid-sodium phosphate buffer (80), was

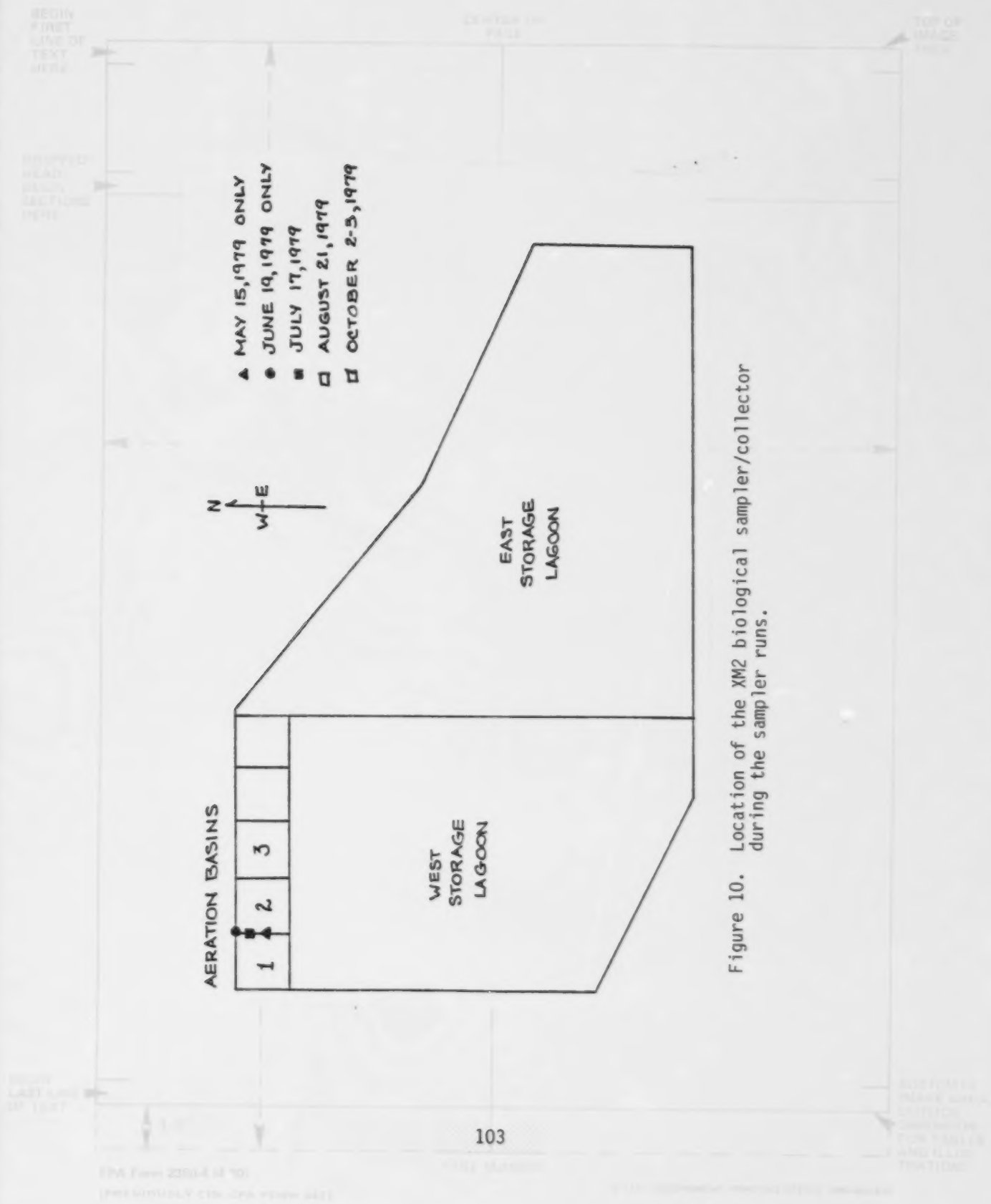


Figure 10. Location of the XM2 biological sampler/collector during the sampler runs.

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
HERE

END OF
FIRST
SECTION
HERE

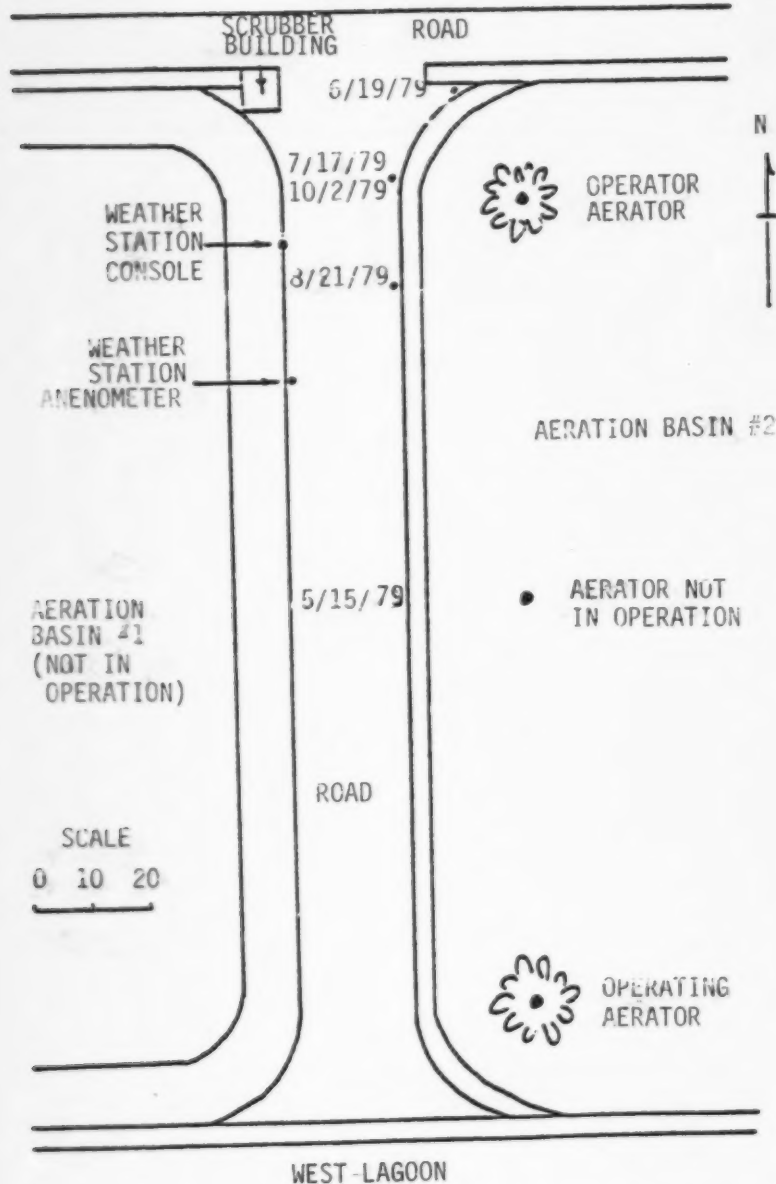


Figure 11. Schematic of aeration basins #1 and #2 showing the location of the XM2 biological sampler/collector during the specified days of the sampling runs.

BEGIN
LAST LINE
OF TEXT

RETURN OF
IMAGE WITH
CUTLINE
DIMENSIONS
FOR TABLES
AND FIGURES
TABLETS

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
PAGE
AREA

DROPPED
HEAD
ROOM
SECTION
HERE

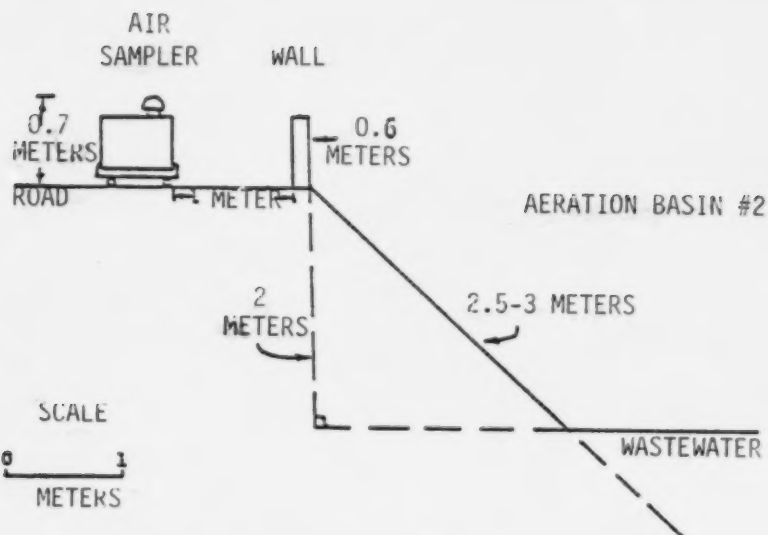


Figure 12. Schematic showing the position of the XM2 biological sampler/collector in relation to the edge of the wastewater.

BEGIN
LAST LINE
OF TEXT

105

WITHIN
PAGE AREA
DO NOT
OVERLAP
TEXT
OR
ILLUSTRATIONS

added. All samples were iced immediately and frozen until assayed at the University of Cincinnati. Sediments of deposited foam, found on the side of the aeration basin and on the road adjacent to it, were collected in sterile beakers using sterile spatulae and forceps. The samples were iced and stored at 10°-12° until assayed.

Sample Processing and Virus Assay

One hundred-ml water and 50-ml foam samples were centrifuged for 30 minutes at 1800-2000 rpm in an International Centrifuge, Size 2, and the supernatant liquid was syringe-filtered through a 10% beef extract (Oxoid Lab Lemco Powder), pH 7.0-pretreated 0.45- μ m and 1.2- μ m membrane filter (Millipore) sandwich in a Millipore Swinnex 47-mm filter holder. The beef extract was prepared in McIlvaine's citric acid-dibasic sodium phosphate buffer, pH 7.0 (7-4). The filters were then eluted with 5 ml of the beef extract. Five to ten ml of the beef extract was added to the centrifuged sediments and mixed with a Vortex mixer. The mixture was allowed to stand overnight at 10-12°C before centrifuging for 30 minutes at 1800-2000 rpm. The supernatant (i.e., sediment eluate) was syringe-filtered through a 10% beef extract, pH 7.0-pretreated 0.45- μ m membrane filter. In addition, air samples in ELH with antibiotics were syringe-filtered and the filters eluted by the same method described for the water and foam samples.

Five-ml aliquots of the water and air sample filtrates and 0.5-ml aliquots of the filter eluates, sediment eluates, and foam filtrates were inoculated into 6-ounce prescription bottles containing the continuous line of BGM cells, derived from primary African green monkey kidney cells. After an incubation period of two hours at 37°C, the bottles were overlaid using the plaque technique of Dulbecco and Vogt (81), as modified by Hsiung and Melnick (82).

In addition, aeration basin raw influent samples were processed by a second method, a modification of the low pH membrane filter adsorption-elution method (83), as previously described (80,84). Samples were clarified by pressure filtration through a 127-mm Gelman Metrigard prefilter and a Millipore 0.22- μ m membrane filter in a Millipore 142-mm stainless steel filter holder. The filter and prefilter had been pretreated with 500 ml of 0.1% Tween 80 and washed with 2 liters of sterile distilled water. After eluting the clarifying filter and prefilter with 3% beef extract (40 ml/liter of sample clarified) and collecting the eluate by pressure filtration, the filter and prefilter were aseptically removed for a second elution in a sterile beaker with enough 3% beef extract to cover. The 3% beef extract had been prepared in McIlvaine's citric acid-dibasic sodium phosphate buffer, pH 7.0 (0.12 g citric acid, 1.34 g NaHPO₄·7H₂O, and 3.0 g beef extract in 100 ml distilled water). The second eluate was poured off and centrifuged for 30 minutes at 1800-2000 rpm prior to filtration through a 0.22- and 1.2- μ m membrane filter (Millipore) sandwich, contained in a Swinnex 47-mm membrane filter holder. These eluates were designated clarifying filter eluates one and two. MgCl₂ (1.2 g/liter of clarified sample) was added to the clarified wastewater sample, and the pH was adjusted to 3 with concentrated HCl. The sample was then pressure filtered through a second 0.22- μ m Millipore filter in a 142-mm holder. After eluting the viruses from this membrane filter for 30 minutes with 3% beef extract (40 ml/liter of clarified sample), the eluate was forced through the filter by air

pressure and collected in a sterile container. The acid filtrate was neutralized to pH 7.0 with NaOH, and a portion (100 ml) was later assayed along with the concentrated sample viruses and clarifying filter eluates 1 and 2. The concentrating membrane filter was then eluted a second time for 30 minutes in a sterile beaker with enough 3% beef extract to cover. The eluate was poured off and filtered through a 0.22- μ m membrane filter in a Swinnex 47-mm filter holder. Five-ml aliquots of the neutralized acid filtrate and 1-ml aliquots of the concentrated sample viruses, concentrating filter eluate, and clarifying filter eluates were inoculated into 6-ounce prescription bottles, containing BGM cells, and assayed as previously described.

Well-isolated plaques were picked from tissue culture bottles inoculated with the July 17, 1979 raw influent sample, processed by the membrane filter adsorption-elution method. After the picked plaques were placed in ELH and inoculated into BGM tube cultures, the cultures were incubated at 37°C for up to 10 days. The cultures were checked daily for cytopathic effect (CPE), and those exhibiting 75% or greater CPE were stored at -70°C until further passage and identification could be done by the Clinical Virology Laboratory in the University of Cincinnati College of Medicine. The enterovirus isolates were typed with Lim-Benyesh-Melnick serum pools.

Sediments of deposited foam, collected from the side of the aeration basin and the road adjacent to it, were weighed and placed in sterile beakers. For every 15 g of solids, 40 ml of 10% beef extract, prepared in McIlvaine's citric acid-dibasic sodium phosphate buffer, pH 7.0, was added to produce a slurry. More extract was added if necessary to allow proper mixing (79). The slurries were mixed for 30 minutes on a magnetic stirrer and centrifuged at 17,100 x g in a Sorvall SS-3 Automatic Superspeed Centrifuge for 30 minutes. The supernatants were filtered through a 0.45- and 1.2- μ m membrane filter sandwich in a Swinnex 47-mm membrane filter holder. The filtrates (i.e., foam sediment eluates) were assayed for viruses, 0.5 ml per bottle, in the usual manner.

Coliphage Assay

Untreated air, water, and foam samples were assayed by the soft agar overlay method (85), using two *E. coli* hosts (ATCC numbers 13706 and 11303). Sample dilutions were made in 0.05 M potassium phosphate buffer (5.17 g KH_2PO_4 and 9.56 g K_2HPO_4 in 2 liters distilled water). Assays using a third *E. coli* host, ATCC number 15597, were also done using the soft agar overlay method described by Safferman and Morris (84). Centrifuged, nonfiltered foam sediment eluates were assayed for coliphage by the same methods. In addition, a portion (≥ 170 ml) of each air sample was syringe-filtered through a 10% beef extract, pH 7.0-pretreated 0.45- μ m membrane filter before assaying by the Kott MPN method (86).

Bacterial Assay

Using Standard Methods Agar, Standard Plate Counts, as described in Standard Methods for the Examination of Water and Wastewater, 14th edition (30), were done on all water and foam samples, as well as the air samples collected in ELH without antibiotics.

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
PAGE
AREA

RESULTS AND DISCUSSION

DROPPED
HEAD
BEGIN
SECTION
HERE

No animal viruses were detected in the air samples collected at the Muskegon County Wastewater Management System Number 1 (Table 46). Animal viruses, found in the water samples (Table 46), decreased as the wastewater progressed through the facility, due to aeration, detention, and sedimentation. Because no viruses were detected in the pump station water just prior to distribution by the irrigation rigs, no air samples were collected in the fields near the rigs. Fourteen viral isolates picked from the July 17, 1979 raw influent sample were identified as follows: two Poliovirus 3, nine coxsackievirus B2, and 3 echovirus 7. One isolate contained an enterovirus which was not typable with the pools, and two did not contain enteroviruses.

Using the centrifugation-filtration method, all raw influent samples were found to contain viruses, ranging from 50 to 400 plaque-forming units (PFU) per liter (Table 47). Most of the viruses recovered were present in the sample supernatant liquids. Viruses were found in the sediment eluates in only two of the samples. No viruses were detected in the filter eluates. In three out of four samples, the centrifugation method recovered higher amounts of virus in the supernatants than in those done by the membrane filter adsorption-elution method. Since the centrifugation-filtration method was done within one week of sample collection, there was less chance of virus adsorption to wastewater solids than in the other method, done at a later date.

Using the membrane filter adsorption-elution method, viruses found ranged from 0 to 456 PFU per liter (Table 47). When the filters were eluted, many of the viruses in the raw influent samples were found adsorbed to the membrane filter and/or the particulates separated out during clarification of the samples. Lack of sample prevented a full-scale examination of all parameters involved in the concentration of the viruses in these wastewater samples by this method.

Aeration basin surface foam and aeration basin and road foam sediment eluates were found to be toxic to the BGM cells. An attempt to separate out the toxic material in the foam, by diluting the sample 1:100 with distilled water and using the membrane filter adsorption-elution concentration procedure for viruses, proved unsuccessful. Therefore, the presence or absence of animal viruses in foam and foam sediments could not be determined.

Although no animal viruses were found, coliphage, present in much larger quantities in the wastewater, were recovered in the air sampled at the aeration basins, using three different *E. coli* hosts (ATCC numbers. 13706, 15597, and 11303). Coliphage levels ranged from 0-9 per cubic meter of air sampled (Table 48).

No coliphage of any type were recovered from the air sample collected on 5/15/79. On this date, the sampler was located about 70-73 meters from the center of the nearest operating surface aerator. On all other runs, the sampler was located 20-25 meters from the center of the nearest operating surface aerator. Since at least one type of phage was recovered from the air samples collected in ELH on each of these runs, the greater distance from aerator to sampler was probably responsible for the absence of coliphage in the 5/15/79 upwind sample.

BEGIN
LAST LINE
OF TEXT

RIGHT MARGIN
AREA
CONTAIN
DISCUSS
FOR THIS IN
AND OTHER
NOTATIONS

TABLE 46. VIRUS RECOVERY FROM MUSKEGON AIR^a AND WATER SAMPLES

Sample date	Air samples (PFU/m ³ air sampled) ^b			Water samples ^c (PFU/liter)			
	Sampler-d position	Filtered supernatant	Filter eluate	Raw influent		Lagoon	
				Filtered supernatant	Sediment eluate	Filtered supernatant	Pump station Filtered supernatant
5/15/79	Upwind ^e	0 ^f	0	50	0	0	0
6/19/79	Downwind	0	0	60	0	40	0
7/17/79	Upwind	0	0	360	40	0	0
8/21/79	Downwind	0	0	267	0	27	0
10/2/79	Downwind	0	0	80	27	14	g
	Upwind ^h	0	0				

^aThe XM2 Biological Sampler/Collector processes 126 cubic meters of air in a 2-hour sampling period.
^bPFU, Plaque-forming units.

^cSamples were assayed by the Centrifugation-Filtration Method.

^dThe sampler was located 20-25 m from the center of the nearest operating surface aerator on all sample dates, except as noted.

^eThe sampler was located 70-73 m from the center of the nearest operating surface aerator on all sample dates, except as noted.

^f0 indicates no viruses detected.

^gNo sample collected.

^hSampling run conducted at night (11:00 P.M. to 3:00 A.M., 10/3/79).

TABLE 47. VIRUS RECOVERY FROM MUSKEGON RAW INFLUENT SAMPLES^a

Sample Date	Centrifugation-Filtration Methods ^b (PPU/liter) ^d				Membrane Filter Adsorption-Elution Methods ^c (PPU/liter)					
	Filtered Supernatant	Filter Eluate	Sediment Eluate	Total	Processed Clarified Filtrate Only	Clarifying Filter Eluate 1 2	Processed Clarified Filtrate	Neutralized Acid Filtrate	Concentrating Filter Eluate	Total
5/15/79	50	0	0	50	Not Done	0	0	0	0	0
6/19/79	60	0	0	60	14	28	0	13	0	41
7/17/79	360	0	40	400	198	← CELLS CONTAMINATED				
8/21/79	267	0	0	267	26	← CELLS DETERIORATED				
10/2/79	80	0	27	107	127	310	42	78	10	456
						14	0	7	0	21 ^e

^aSamples were stored at -70°C until assay could be done. The centrifugation-filtration method was performed within one week of the return to Cincinnati. The adsorption-elution method was done at a later date.

^bTen percent beef extract (Oxoid Lab Lencoc powder) in McIlvaline's citric acid-dibasic sodium phosphate buffer, pH 7.0, was used for all elutions in this method.

^cThree percent beef extract (Oxoid Lab Lencoc powder) in McIlvaline's citric acid-dibasic sodium phosphate buffer, pH 7.0, was used for all elutions in this method, except as noted.

^dPPU, Plaque-forming units.

^e0 indicates no viruses recovered.

^fThree percent beef extract (Oxoid Lab Lencoc powder) in McIlvaline's citric acid-dibasic sodium phosphate buffer, pH 8.0, was used for all elutions.

TABALE 48. COMPARISON OF THE COLIPHAGE RECOVERY FROM MUSKEGON AIR SAMPLES^a USING THE SOFT AGAR OVERLAY METHOD AND KOTT'S MPN METHOD

Sample date	Sampler position ^b	Collecting liquid	Coliphage recovered using phage host:					
			E. coli 13706 (strain C, ox-174 host)		E. coli 15597 (C-3000, f2 host)		E. coli 11303 (strain B, T1-7 host)	
			Overlay method ^c	MPN method ^d	Overlay method ^c	MPN method ^d	Overlay method ^c	MPN method ^d
5/15/79	Upwind ^e	ELH ^f	0 ^g	0	0	0	0	0
6/19/79	Downwind	DW ^h	0	0	0	0	0	0
	Downwind	ELH	2.08	9.0	0	0.033	0	0
7/17/79	Upwind	ELH	0	0.42	0	0	0	0
8/21/79	Downwind	ELH	1.59	2.22	1.59	0.13	0	0.11
10/2/79	Downwind	ELH	0	2.04	0.86	0.85	0	0.21
	Upwind ⁱ	ELH	0.79	1.17	0	0.19	0	0.31

^aThe XM2 Biological Sampler/Collector processes 126 cubic meters of air in a 2-hour sampling period.

^bThe sampler was located 20-25 m from the center of the nearest operating surface aerator on all sampling dates, except as noted.

^cValues are expressed as plaque-forming units per cubic meter of air samples.

^dValues are expressed as Most Probable Number per cubic meter of air sampled.

^eThe sampler was located 70-73 m from the center of the nearest operating surface aerator.

^fSterile Earle's Lactalbumin Hydrolysate without antibiotics.

^g0 indicates no coliphage detected.

^hSterile distilled water.

ⁱSampling run conducted at night (11:00 P.M. to 3:00 A.M., 10/3/79).

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTION
HERE

More E. coli 13706 coliphage were recovered than either of the other two types. The largest amount of coliphage (9 MPN/m³ air sampled, June 19, 1979 sample) was recovered in ELH, using the MPN test and this host. During this air sampling run, the sampler was positioned downwind approximately 90% of the time (Table 49), in a fine mist that could be felt. Using this host, all three downwind MPN levels from samples collected in ELH were higher than the three upwind samples. The results were more erratic with the overlay method, with phage recoveries from 2 downwind samples and 1 upwind sample. However, the upwind sample was taken at night (October 2-3, 1979) when environmental stresses (high temperature, solar radiation, increased wind velocity, dessication, etc.) would be less than encountered during the day.

Using E. coli 15597, coliphage were recovered in the MPN test from all three downwind samples collected in ELH. The only upwind sample in which they were detected was the sample collected at night, when environmental conditions would be more favorable for survival than during the day. Coliphage were detected only in downwind samples with the overlay method.

Using E. coli 11303, coliphage were recovered only using the MPN test. The upwind night sample (October 2-3, 1979) had a slightly higher level than the downwind sample collected earlier that same day. Because these coliphage were present in lower amounts in the wastewater than the other two types, lower amounts would be expected in the aerosols. Although the E. coli 11303 levels were lower than the E. coli 13706 levels, they were very similar to those of E. coli 15597 coliphage. In only one case was the E. coli 15597 coliphage level considerably higher. This may indicate that E. coli 15597 coliphage have a greater die-off rate due to environmental stresses and/or collection and freezing than E. coli 11303 coliphage.

Kott's MPN method was found to be more sensitive and consistent in detecting low levels of coliphage in the air samples than the soft agar overlay method. No E. coli 11303 coliphage were recovered using the overlay method, but in 3 out of 7 cases, they could be recovered with the MPN method. This is probably due to the growth of the coliphage by overnight incubation with the host prior to assay on freshly seeded plates.

All three types of coliphage were found in all raw influent wastewater samples, but levels decreased in the lagoon and pump station samples (Table 50). In general, the quantity of each type of coliphage present in Muskegon foam (Table 51) and wastewater samples can be expressed in the following relationship:

$$\boxed{\begin{array}{c} \text{NUMBER OF} \\ \text{E. COLI} \\ 13706 \\ \text{COLIPHAGE} \end{array}} \geq \boxed{\begin{array}{c} \text{NUMBER OF} \\ \text{E. COLI} \\ 15597 \\ \text{COLIPHAGE} \end{array}} > \boxed{\begin{array}{c} \text{NUMBER OF} \\ \text{E. COLI} \\ 11303 \\ \text{COLIPHAGE} \end{array}}$$

Concentrations of coliphage in foam samples varied, depending on the amount of foam collected and the type and amount of diluent, but the undiluted foam sample, assayed using E. coli 13706, 15597, and 11303, contained 2.85×10^3 , 1.8×10^3 , and 4.0×10^2 PFU per ml coliphage, respectively. Although the

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
5 1/4" MINIMUM
DIMENSION
FOR TABLES
AND ILLU-
STRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTION
HERE

TABLE 49. SUMMARY OF WEATHER CONDITIONS AT MUSKEGON DURING AIR SAMPLING RUNS

Sample date (and time)	Sampler position	Prevailing wind direction	Range of wind velocity	Temperature range (°F)	Range of relative humidity (%)	Average daily ^a barometric pressure (inches of Hg)	Additional weather observations
5/15/79 (9:42 AM to 1:50 PM)	Upwind about 90% of the time	From the NW	10-20 mph with strong gusts to 30 mph	60-67	39-56	29.48	Partly cloudy early with warm, sunny and bright conditions later; nearby irrigation rigs may have had some effect on sample.
6/19/79 (2:00 to 8:10 PM)	Downwind about 80% of the time, in the mist	From the SE	10-20 mph with many gusts	81-85	45-52	29.43	Sunny, hot, windy, bright with slight haze; irrigation rigs had no effect on sample.
7/17/79 (3:45 to 7:50 PM)	Upwind about 95% of the time	From the NW	0-10 mph, in- creasing to 10-20 mph later	76-81	38-52	29.58	Sunny, bright, hot, some clouds; irrigation rigs had a questionable effect on sample.
8/21/79 (10:47 AM to 2:53 PM)	Downwind about 90% of the time, in the mist	From the NE to SE	0-13 mph, occasional gusts	79-83	53-63	29.40	Sunny, bright, hot, slight haze, few clouds; irrigation rigs had little effect on sample.
10/2-3/79 (4:11 to 8:00 PM)	Downwind almost all of the time	From the E to NE	0-10 mph, occasional gusts	69-72	63-74	29.04	Heavy rain in very early morning, cloudy, overcast, and warm later; irrigation rigs may have had some effect on sample.
(11:00 PM to 3:00 AM)	Upwind most of the time	From the N to NW	Almost none	About 50	74-79	29.04	Cloudy, cold, some fog.

^aTaken from the monthly data sheets of the National Weather Service office at the Muskegon County Airport.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
FOR TABLE
AND ILLU-
STRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
PAGE

DROPPED
HEAD;
BEGIN
SECTION
HERE

TABLE 50. COLIPHAGE RECOVERY FROM MUSKEGON WATER SAMPLES USING THE SOFT AGAR OVERLAY METHOD

Sample Site	Phage Host Used (E. coli ATCC No.)	Coliphage Recovered (PFU/liter) ^a From Sample Taken On:					
		4/5/79	5/15/79	6/19/79	7/17/79	8/21/79	10/2/79
Pavation Pond (Raw Effluent)	13706	1.45x10 ⁵	2.05x10 ⁵	5.05x10 ⁵	3.68x10 ⁵	1.84x10 ⁵	5.53x10 ⁵
	15597	1.22x10 ⁵	1.11x10 ⁵	8.80x10 ⁴	8.85x10 ⁴	4.90x10 ⁴	1.32x10 ⁵
	11303	b	4.50x10 ³	3.25x10 ³	1.05x10 ⁴	8.80x10 ³	8.03x10 ⁴
West Lagoon	13706	4.77x10 ⁴	5.00x10 ²	1.00x10 ³	0 ^c	3.00x10 ³	3.00x10 ³
	15597	6.45x10 ⁴	2.00x10 ³	0	0	3.30x10 ²	0
	11303	b	0	0	1.00x10 ³	0	0
Pump Station (Before Nitrogen Added)	13706	d	d	5.00x10 ²	0	d	d
	15597	d	d	0	0	d	d
	11303	d	d	0	0	d	d
Pump Station (After Nitrogen Added)	13706	d	d	1.00x10 ³	1.00x10 ³	6.00x10 ³	d
	15597	d	d	0	5.00x10 ²	0	d
	11303	d	d	0	0	0	d

^aPFU, Plaque-forming units.

^bNot done.

^c0 indicates no coliphage detected.

^dNo sample collected.

TABEL 51. COLIPHAGE RECOVERY FROM MUSKEGON FOAM SAMPLES USING THE SOFT AGAR OVERLAY METHOD

Sample Date	Type Suspending Liquid	Total Volume ^a (ml)	Coliphage Recovered (PFU/ml) ^b Using Phage Host:		
			E. Coli 13706 (Strain C, 4x-174 Host)	E. Coli 15597 (C-3000, f2 Host)	E. Coli 11303 (Strain B, T1-7 Host)
5/15/79	ELH ^c	50	13	20	1.0
6/19/79	DW ^d	50	37.3	0 ^e	0
7/17/79	ELH	100	363	514	29.5
8/21/79	10% BE, pH 7.0 ^f	150	927	705	37.5
	ELH	150	390	150	16.83
10/2/79	None (Undiluted)	g	2850	1800	400
	ELH	150	347	281	16.17

^a Foam samples were collected in a sterile beaker, allowed to settle, and were made up to a final volume with the chosen suspending liquid. Assays were done on frozen samples.

^b PFU, plaque-forming units.

^c Sterile Earle's Lactalbumin Hydrolysate without antibiotics.

^d Sterile distilled water.

^e 0 indicates no coliphage recovered.

^f 10% Beef extract in McIlvaine's citric acid-dibasic sodium phosphate buffer, pH 7.0.

^g Not applicable.

eluate of the foam sediment from the road adjacent to the aeration basin contained no coliphage, the eluate of the foam sediment inside the aeration basin contained 3.7×10^4 PFU E. coli 15597 coliphage per gram of sediment.

Bacteria, ranging from 86-7143 colony-forming units (CFU) per cubic meter of air sampled, were recovered from the air samples (Table 52). The largest amount (August 21, 1979 sample) was recovered during a run when the sampler was positioned downwind approximately 90% of the time in a mist that could be felt. The next largest (5254 CFU/m³ air sampled, May 15, 1979 sample) was obtained during a run when the sampler was upwind most of the time. However, the three irrigation rigs north of the aeration basin (and, therefore, upwind of the sampler) were operating during this run, and there were frequent large gusts of wind, up to 30 mph or more. In addition, both samples were collected, for the most part, in the morning when aerosolized microorganisms would have less exposure to heat, desiccation, and solar radiation than at later times in the day. The upwind night run, collected on October 2-3, 1979, was slightly higher than the downwind run done earlier that same day. This is probably due to the much cooler temperatures and the absence of solar radiation. Since all of the plate counts were done on frozen air samples, some bacteria were probably lost. The lowest amounts recovered (upwind and downwind, October 2-3, 1979) were probably due, in part, to the cleansing action of a heavy early morning rain on the air.

ELH appeared to be a better collection liquid than distilled water. The sample collected in ELH on June 19, 1979 (Table 52) was found to contain nearly 70% more bacteria than that collected in distilled water that same day. No coliphage (Table 48) were recovered from the distilled water sample, but 2 of the 3 types of coliphage were recovered from the sample collected in ELH. ELH appeared to help bacteria and coliphage survive the stresses of sample collection and freezing.

As in the case with virus and coliphage levels, total bacterial counts decreased as the wastewater progressed through the treatment facility to the irrigation rigs (Table 53). In 4 out of 5 cases, standard plate counts on diluted foam samples were only one log lower than those of the corresponding raw influent samples (Table 54). One additional diluted foam sample was found to contain the same amount of bacteria, and an undiluted sample contained 7.65×10^7 CFU/ml, which is 3.5 logs higher than its corresponding raw influent sample. Considering the fact that the diluted samples contained 10-20% foam or less, it appears that the aeration basin foam may either concentrate bacteria from the wastewater or may exert a protective effect on bacteria in the frozen samples.

The advantages and disadvantages of the XM2 Biological Sampler/Collector are listed in Table 55. Although particles $< 2 \mu\text{m}$ in diameter were not retained by the sampler, coliphage were collected from the aerosols at this site. Because of this and because some coliphage are similar in size to some enteroviruses (e.g., polio is similar to f2 coliphage) (87), it appears that the sampler would collect animal viruses if the aerosol levels were large enough and/or if the sampling periods were long enough.

TABLE 52. RECOVERY OF BACTERIA FROM MUSKEGON AIR SAMPLES^a

Sample date	Sampler ^b position	Standard plate count ^c (CFU/ml sample) ^d	CFU/m ³ air sampled ^e
5/15/79	Upwind ^f	3310	5254 (149)
6/19/79	Downwind	127	212 (6.0)
	Downwind ^g	64	126 (3.6)
7/17/79	Upwind	124	305 (8.6)
8/21/79	Downwind	4500	7143 (202)
10/2-3/79	Downwind	33	86 (2.4)
	Upwind ^h	42	99 (2.8)

^aThe XM2 Biological Sampler/Collector processes 126 cubic meters of air in a 2-hour sampling period.

^bThe sampler was located 20-25 m from the center of the nearest operating surface aerator on all sample dates, except as noted.

^cBacterial assays were done on frozen samples which had been collected in sterile Earle's Lactalbumin Hydrolysate without antibiotics, except as noted.

^dCFU, Colony-forming units.

^eCFU/ft³ air samples shown in parenthesis.

^fThe sampler was located 70-73 m from the center of the nearest operating surface aerator.

^gSample was collected in sterile distilled water.

^hThis sampling run was conducted at night (11:00 P.M., 10/2/79, to 3:00 A.M., 10/3/79).

TABLE 53. STANDARD PLATE COUNTS OF MUSKIEON WATER SAMPLES^a
Bacterial Counts (CFU/ml)^b At Those Sampling Sites:

Sample Date	Aeration Basin Raw Influent	West Lagoon	Pump Station	
			Before Nitrogen Addition	After Nitrogen Addition
4/5/79	4.65×10^5	1.10×10^6	c	c
5/15/79	4.73×10^5	3.25×10^4	c	c
6/19/79	1.76×10^6	1.94×10^5	6.00×10^3	1.83×10^4
7/17/79	3.19×10^5	4.10×10^2	3.75×10^2	5.50×10^2
8/21/79	2.75×10^7 ^d	3.50×10^{3d}	c	4.60×10^{3d}
	3.35×10^5	e	c	1.11×10^3
10/2/79	8.90×10^{6df}	6.73×10^{3df}	c	c
	1.67×10^4	2.15×10^2	c	c

^a Assay done in Cincinnati, Ohio, on frozen samples, except as noted.

^b CFU, Colony-forming units.

^c No sample collected.

^d Assay done in Muskegon, Michigan, as soon as possible after sample collection.

^e Not done.

^f Plates counted at 90 hours.

TABLE 54. COMPARISON OF STANDARD PLATE COUNTS^a ON MUSKEGON FOAM AND AERATION BASIN RAW INFLUENT SAMPLES

Sample date	Suspending liquid of foam	Final volume ^b of foam sample (ml)	Bacterial counts (CFU/ml) ^c	
			Foam	Raw Influent
5/15/79	ELH ^d	50	1.19×10^4	4.73×10^5
6/19/79	DW ^e	50	3.9×10^3	1.76×10^6
7/17/79	ELH	100	2.55×10^4	3.19×10^5
8/21/79	10% BE, pH 7.0 ^f	150	3.25×10^5	3.35×10^5
	ELH	150	4.8×10^4	
10/2/79	None (undiluted)	g	7.65×10^7	1.67×10^4
	ELH	150	3.46×10^3	

^aAssay was done on frozen raw influent and foam samples in Cincinnati, Ohio.

^bFoam samples were collected in a sterile beaker, allowed to settle, and were made up to a final volume with the chosen suspending liquid.

^cCFU, Colony-forming units.

^dSterile Earle's lactalbumin hydrolysate without antibiotics.

^eSterile distilled water.

^f10% beef extract in McIlvaine's citric acid-dibasic sodium phosphate buffer, pH 7.0.

^gNot applicable.

TABLE 55. THE ADVANTAGES AND DISADVANTAGES OF THE XM2 BIOLOGICAL SAMPLER/COLLECTOR

Advantages

1. The XM2 Biological Sampler/Collector processes a large volume of air (1050 liters/minute).
2. The air unit can be removed from the sampler for ethylene oxide sterilization.
3. Many of the particles in the respirable range can be collected.
4. Particles are collected in liquid, thereby minimizing the dehydration of airborne viruses and bacteria and increasing their chances of survival.
5. Particles are collected by a combination of scrubbing and impingement in the wet collector of the sampler.
6. No arcing or other electrical problems occur with this sampler.

Disadvantages

1. Sterilization procedures cannot be done quickly.
2. The air unit cannot be autoclaved.
3. Sterilization cannot be done in the field.
4. The sampler is complicated, and the adjustment of the peristaltic pump is difficult. Considerable time is necessary to become familiar with the sampler.
5. The sampler does not collect the particles $<2 \mu\text{m}$ or $>12 \mu\text{m}$ in diameter.
6. Two people are needed to lift the cumbersome and heavy sampler in and out of the transporting vehicle. However, it can be moved quite easily once it is situated on a wheeled platform at the sampling site.
7. An adequate supply of electricity is needed at the sampling site.

Possible explanations for the failure to recover animals viruses are the following:

1. Raw influent virus levels may be too low to produce the aerosol levels necessary to be collected with this sampler.
2. Viruses may not be aerosolized due to adsorption to wastewater particulates and/or entrapment by the blanket of foam on the surface of the aeration basin.
3. Viruses may not survive aerosolization by the aerators, dessication and debilitation by environmental factors, and damage during the passage through the sampler air unit and tubing to the collection liquid.
4. Viruses may be present in the particles not retained by the XM2 Sampler; i.e., in those $< 2 \mu\text{m}$ and $> 12 \mu\text{m}$ in diameter. In order to collect these, the sampler design would have to be altered, thereby affecting the air flow through the air unit and the separation of the $2\text{-}12 \mu\text{m}$ particles. Further, as currently designed the air passages for these particles ($< 2 \mu\text{m}$ and $> 12 \mu\text{m}$) cannot be sterilized.
5. A longer sampling period (perhaps several days to several weeks) may be necessary to prove the presence or absence of animal viruses. An alternative to this would be to pool air samples from multiple samplers and/or sampling runs.
6. Cell systems, other than BGM, may give better recovery of viruses at this site.
7. Loss of virus may have occurred during storage and assay procedures. For example, viruses may adhere to sample containers and pipettes, become entrapped on membrane filters, in spite of pre-treatment, or may die during freezing and thawing of the samples.

FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTIONS
HERE

SECTION 8

BACTERIAL AEROSOL ENUMERATION AND IDENTIFICATION AT A
WASTEWATER SPRAY IRRIGATION SYSTEM

The purpose of this project was to collect, enumerate and identify bacteria in aerosols at the Muskegon County, Michigan Wastewater Management System Number 1. The description of this facility is presented in Section 7.

MATERIALS AND METHODS

Air Sampling

Aerosols are particulate materials in either solid or liquid form and may also include gases and vapors that are adsorbed or contained in airborne particles or liquid droplets. Inhalation is a possible route of infection because viruses and most pathogenic bacteria are in the respirable size range. The health hazard posed by aerosolized particles depends in part on their ability to deposit in the lungs. The most important factor in lung deposition is the size of the particle. The particle size is usually expressed as mass median or aerodynamic diameter. The aerodynamic diameter is a function of both the physical diameter and the density of the particle. It is defined as the diameter of a unit density sphere having the same settling velocity as the particle in question of whatever shape and density. Usually particles with an aerodynamic diameter greater than $30\text{ }\mu\text{m}$ do not enter the nasal passage, those with an aerodynamic diameter ranging from $5\text{--}30\text{ }\mu\text{m}$ are deposited in the naso-pharyngeal region. Particles ranging in aerodynamic diameter from $1\text{--}5\text{ }\mu\text{m}$ are usually deposited in the tracheobronchial region by sedimentation. Particles less than $1\text{ }\mu\text{m}$ in aerodynamic diameter are deposited in the pulmonary or alveolar region by diffusion. It is the last category of particles that may constitute a health hazard by inhalation. Particles deposited in the tracheobronchial region can be removed by muco-ciliary action (spiral movement of the mucus by ciliated epithelium) toward the trachea and pharynx where the material is swallowed or expectorated. The swallowed particles then may pose a health hazard via ingestion by exposing the gastrointestinal tract to the pathogens.

The Andersen six-stage cascade impactor (87,88) was used in this project (Figure 13). The Andersen air sampler collects and separates particles into different size ranges and thus provides a good size distribution of the particles. The Andersen sampler is used only when particle concentrations are high, since it has a limited sampling range of 28.3 liters/

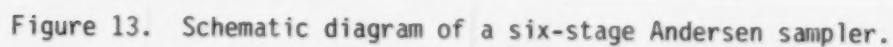
BOTTOM OF
IMAGE AREA
OUTSIDE
VISION
FOR TABLES
AND ILLUSTRATIONS

BEGIN
LAST LINE
OF TEXT

3/8"

122

PAGE NUMBER



min. The perforations become smaller with each succeeding descending stage, so that as a pump pulls one cubic foot of air per minute through the sampler the particles will be sized aerodynamically such that the respirable (stages 3-6) versus nonrespirable (stages 1-2) particles can be separated.

The samplers were loaded using aseptic technique and autoclaved between use to minimize carry-over contamination. The closed samplers were then moved to the sampling sites.

Sampling Sites

The sampling sites were chosen to evaluate the contribution of various treatment units and to determine aerosol levels in worker areas. Upwind and downwind samples were taken in the irrigated fields and at the aeration basins throughout the growing season. Air samples were also taken at the pump stations, the farm, the administration building and also at the nearby County Road Commission just outside the Wastewater Management System (Figure 14). The samplers were run at one cubic foot per minute for ten, five, or two minutes depending on the number of colony forming units (CFU) found in previous runs.

Enumeration and Gram-Negative Bacteria Isolation and Identification

The primary cultivation medium used in the Andersen sampler was Trypticase Soy Agar (TSA), a nutritional medium. The exposed plates were incubated for 24 hours at 35°C. The colonies on these plates were counted, and plates three through six were replicated using velvet onto two more TSA plates which were again incubated for 24 hours at 35°C. These submaster plates were then each replicated onto three different media: sheep blood and MacConkey's agars for identification; and KF Streptococcal, M-endo, M-FC, and XLD agars for enumeration of fecal indicator organisms (30). The colonies which grew on MacConkey's agar were considered to be gram-negative colonies, and the API-20E system of 21 biochemical tests were used to identify the isolates. The colonies which did not grow on MacConkey's, but did grow on sheep blood agar, were considered to be gram-positive, tentatively, and worked up in another fashion. A flow diagram for this enumeration, isolation and identification is presented in Figure 15.

Gram-Positive Bacteria Identification

There was an initial problem with the identification of the gram-positive bacteria, since the soil organisms present in the air were interfering with the tests. This interference was avoided by using selective media to eliminate the soil bacteria. Thus, KF-Streptococcal Agar was used to select out for streptococci, and Mannitol Salt Agar was used to select out for staphylococci (30).

B and O disks were used to identify group A beta streptococcus and *Streptococcus pneumoniae*, respectively, after they showed rings of inhibition on Sheep Blood Agar at 35°C for 24 hr. Any negative cultures were streaked onto Bile Esculine Agar and incubated for 35°C for 3 days, checking daily.

BEGIN FIRST LINE OF TEXT HERE

CENTER OF PAGE

TOP OF IMAGE AREA

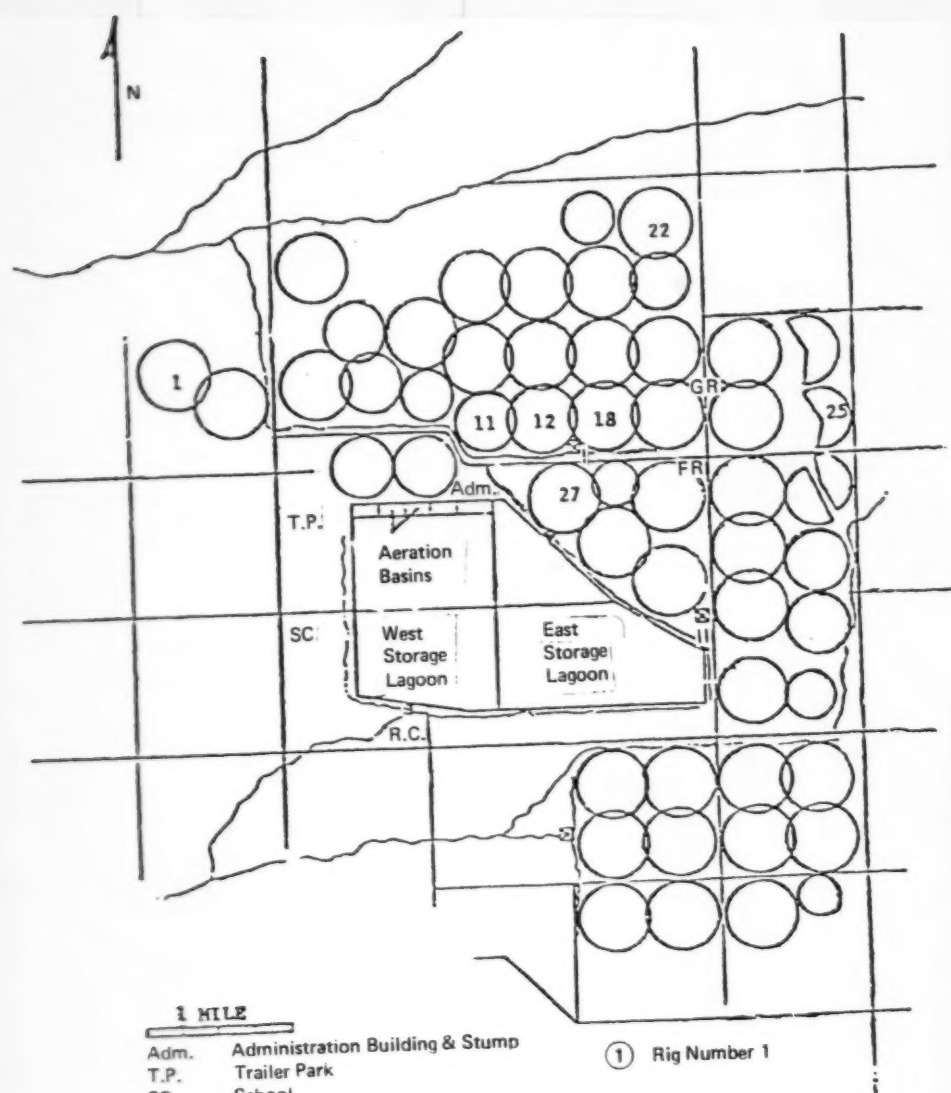
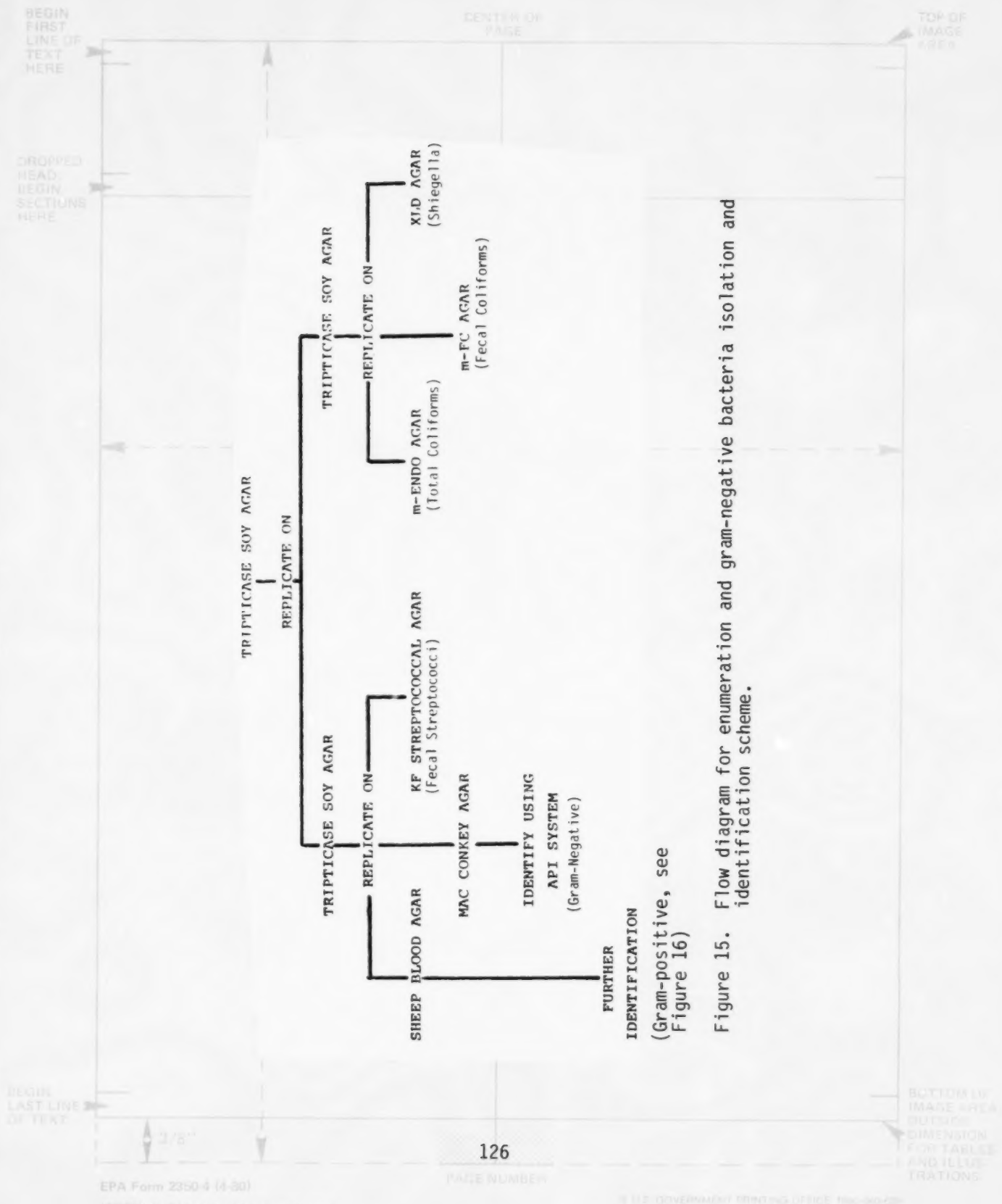


Figure 14. Map of Muskegon Wastewater Management System no. 1 showing the air sampling sites.

BEGIN LAST LINE OF TEXT HERE

BOTTOM OF IMAGE AREA, OUTSIDE DIMENSIONS FOR TABLES AND ILLUSTRATIONS



(Gram-positive, see Figure 16)

Figure 15. Flow diagram for enumeration and gram-negative bacteria isolation and identification scheme.

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD;
BEGIN
SECTIONS
HERE

Any Bile Esculine positive cultures were inoculated into Brain Heart Infusion Broth and incubated at 35°C for 24 hr. The 24 hr. culture was inoculated into Brain Heart Infusion Agar with 6.5% salt and incubated for 3 to 7 days at 35°C. If there was growth here, the culture was considered to be either Streptococcus faecalis or faecium.

Any culture which grew on the Mannitol Salt Agar was inoculated into Brain Heart Infusion Broth at 35°C for 24 hr. A drop of this culture was added to 0.49 ml of rabbit coagulase plasma, covered, and incubated in a 37°C water bath for 24 hr. If the tube was clotted, the culture was called Staphylococcus aureus.

A flow diagram of the replication and identification scheme for gram-positive bacteria is shown in Figure 16.

RESULTS

The proportions of the respirable CFU recovered on the various media at the treatment plant sites sampled and the mean respirable CFU at these sites are shown in Table 56.

The downwind air from the aeration basin area contained the highest numbers of colonies on M-Endo, M-FC, and MacConkey agars, and Colony Forming Units (CFU) in the respirable size range. Although the samples in the fields were taken upwind and downwind, there appeared to be a definite cross-over of air from another or the same rig. This can be seen in the fact that the upwind and downwind rig sample values were closer and not as different as the values found upwind and downwind of the aeration basins. The background values taken in the fields after the rigs had been out of operation for several weeks were much lower. The proportional number of the various respirable bacterial organisms identified in the samples grouped according to location are presented in Figures 17 to 21. The data presented in these figures represent different number of samples collected and because each sample collected represented a particular volume of air, the total volume of air, M^3 , from which the identifications were made is included in each figure.

There was found to be an increase in the number of bacteria found in the surrounding air as a result of the wastewater site. The increased numbers of bacteria found downwind of the aeration basin and the irrigation rigs in the fields, suggested that these areas were sources of bacterial contamination of the air. The population of aerosolized bacteria downwind of the aeration basin and the irrigated field rigs did have a higher percentage of gram-negative bacilli than the upwind samples, suggesting that the areas were also a source of gram-negative bacilli.

The population of aerosolized bacteria in the downwind air had a higher percentage of fecal-indicating bacteria than the control samples. In addition, the population of aerosolized bacteria in downwind samples did have a higher percentage of pathogenic bacteria (defined as gram-negative bacteria and gram-positive cocci) except for Staphylococcus aureus and

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA,
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

3/8"

127

PAGE NUMBER

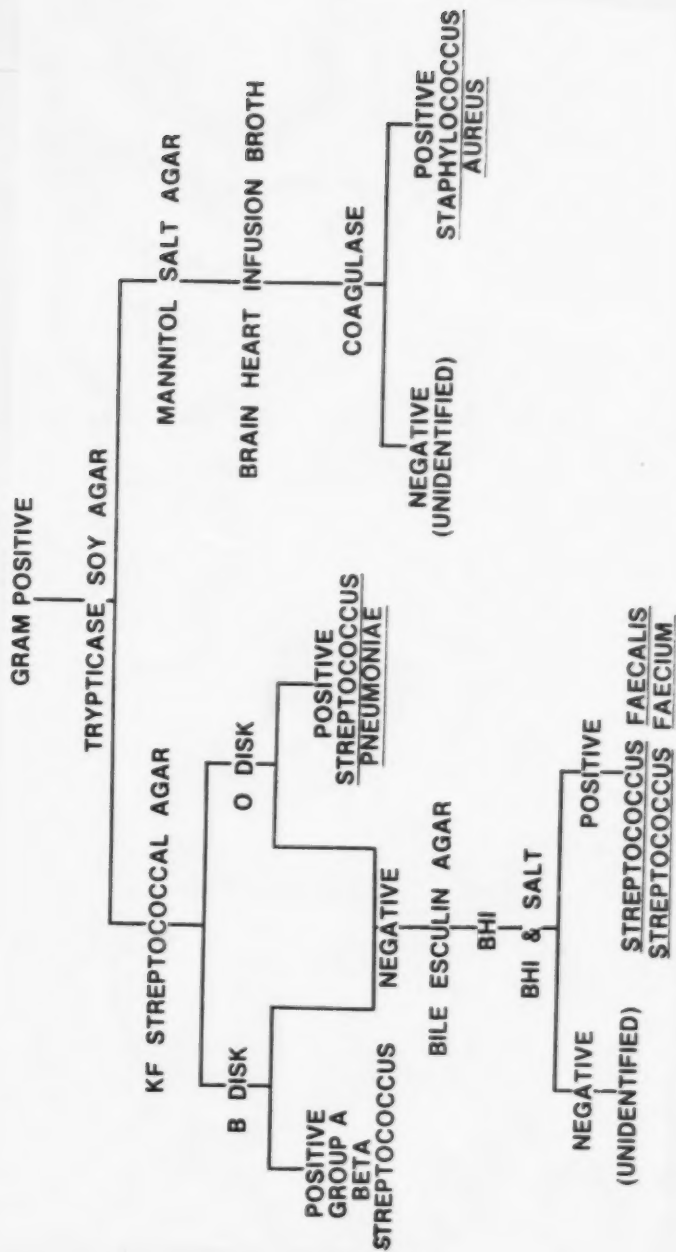


Figure 16. Flow diagram of the replication and identification scheme for gram-positive bacteria.

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD;
BEGIN
SECTIONS
HERE

TABLE 56. ENUMERATION OF RESPIRABLE COLONY FORMING UNITS
RECOVERED AT VARIOUS SITES

		Means of total colony forming units of respirable size that were followed per cubic meter of air			
		Media/type of bacteria			
Source*		M-Endo/total coliforms	M-FC/fecal coliforms	McConkey/ gram-negative	TSA/ total
Aeration basin (1) upwind**		51	15	140	490
(1) downwind**		1100	1600	2200	2800
Field rigs (18.3) upwind		21	28	110	660
(18.3) downwind		35	35	290	700
(18.3) background		0	0	70	320
East & west lagoon (1)		0	100	100	380
Farm storage (200) 6-1/2"		0	0	18	230
Pump station I (6)		0	0	35	620
Pump station II (6) 8-1/8"		0	18	100	350
Administration Building (400)		0	35	70	500
Road Commission (400)		0	0	11	370
Trailer park (200)		18	18	260	380
Grainery (400)		0	70	0	320
Inn (17500)		0	0	70	240
School (440)		100	0	140	350

*Approximate distance (meters) from edge of nearest source (aeration basin
or spray boom - see Figure 14).

**One sample collected at 18.3 meters was in the same range as the others.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA;
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

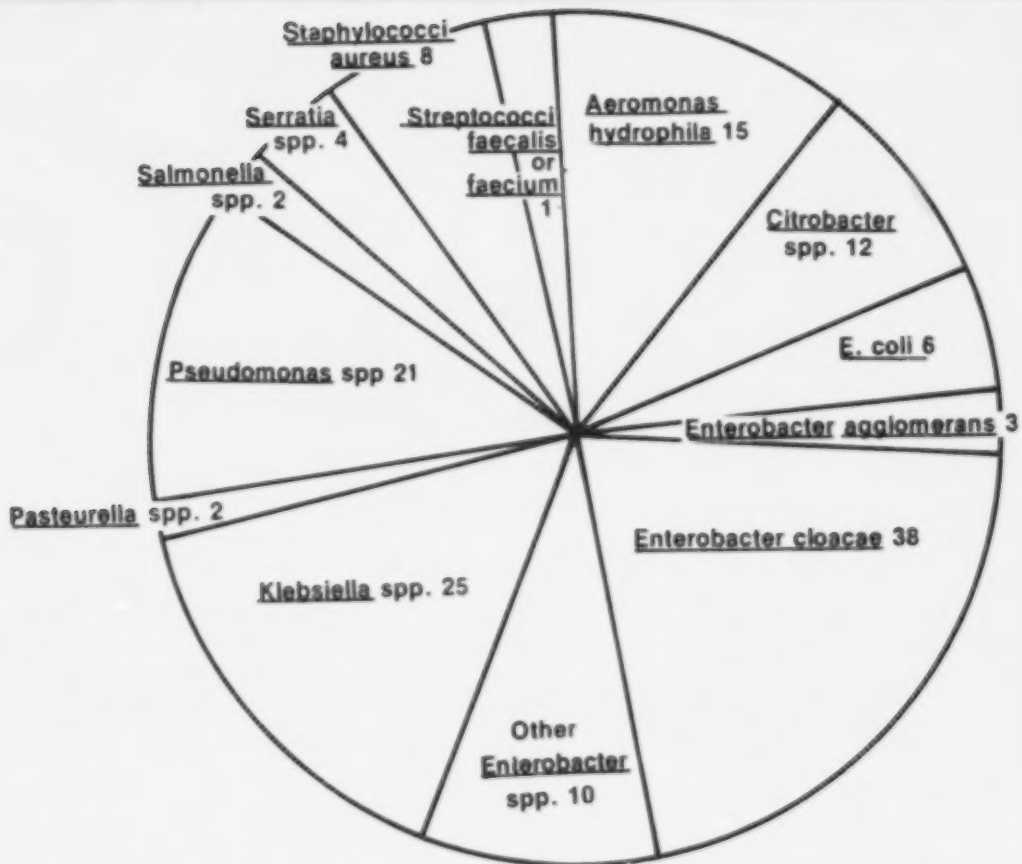


Figure 17. Relative proportions of respirable bacteria identified downwind of aeration basin in 2.06 cubic meters of air.

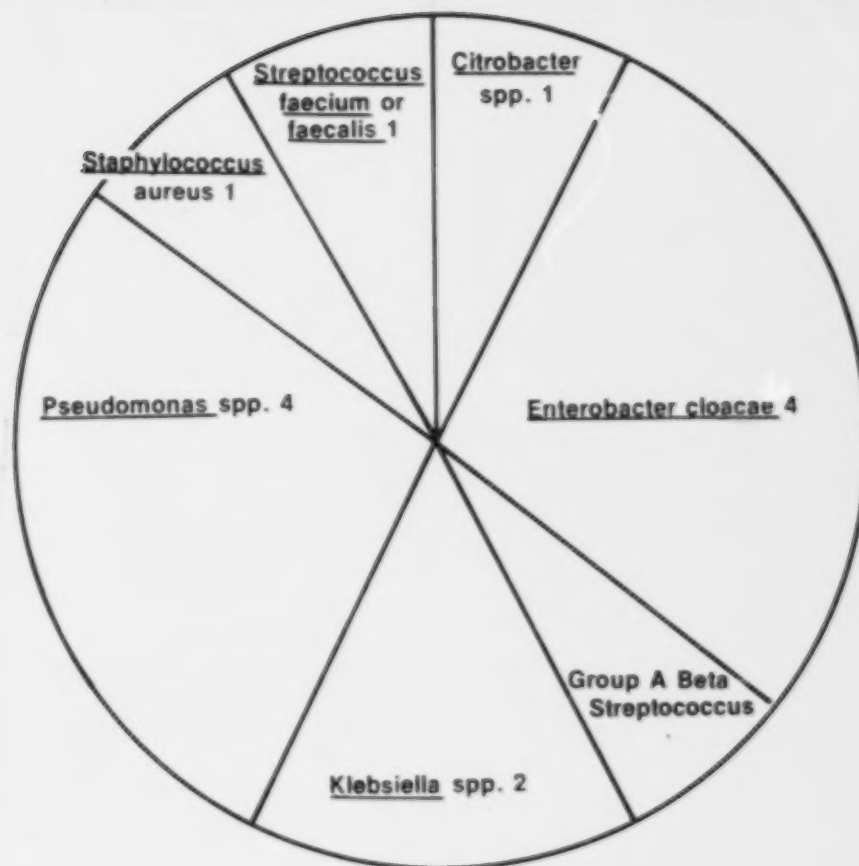


Figure 18. Relative proportions of respirable bacteria identified upwind of aeration basin in 2.01 cubic meters of air.

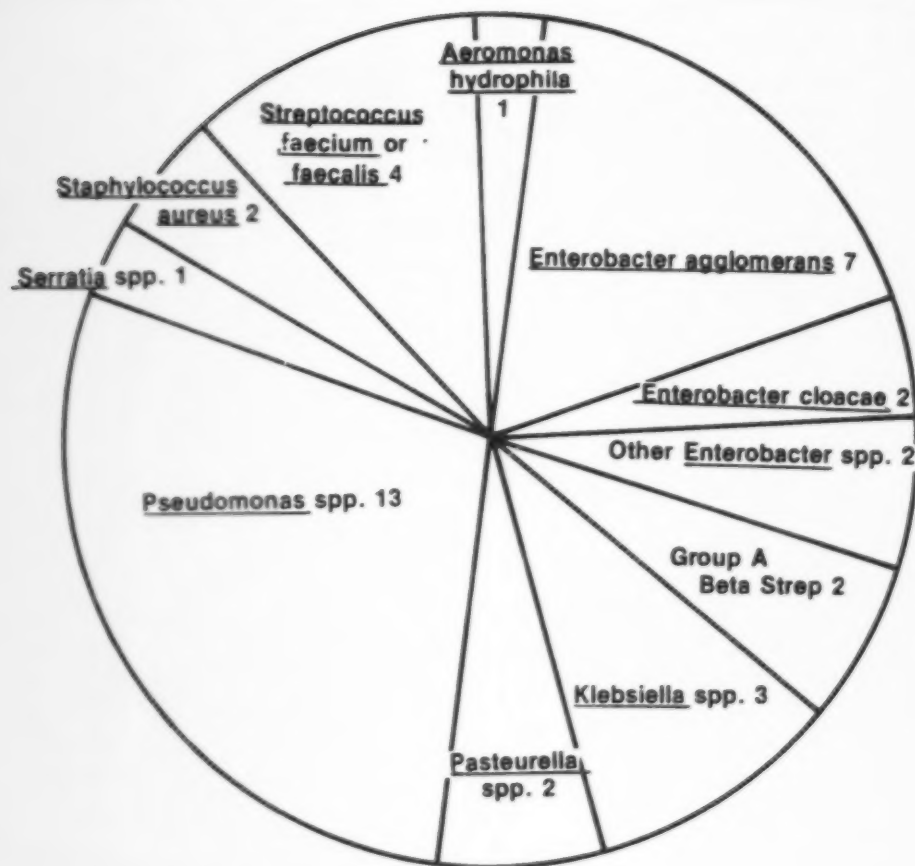


Figure 19. Relative proportions of respirable bacteria identified downwind of field rigs in 3.97 cubic meters of air.

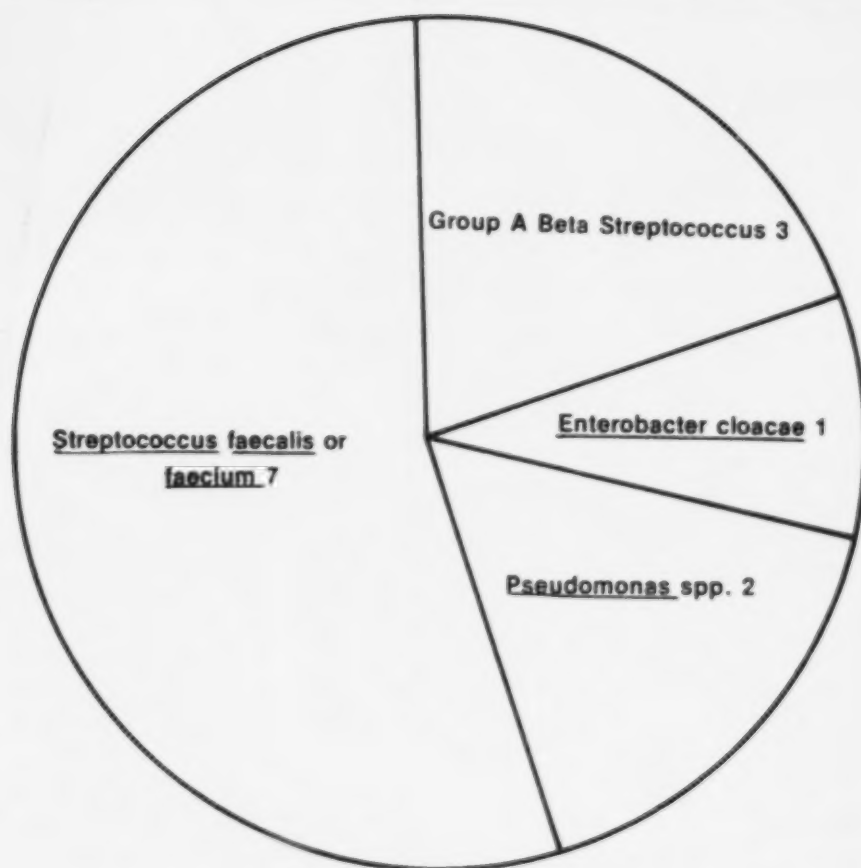


Figure 20. Relative proportions of respirable bacteria identified upwind of field rigs in 3.97 cubic meters of air.

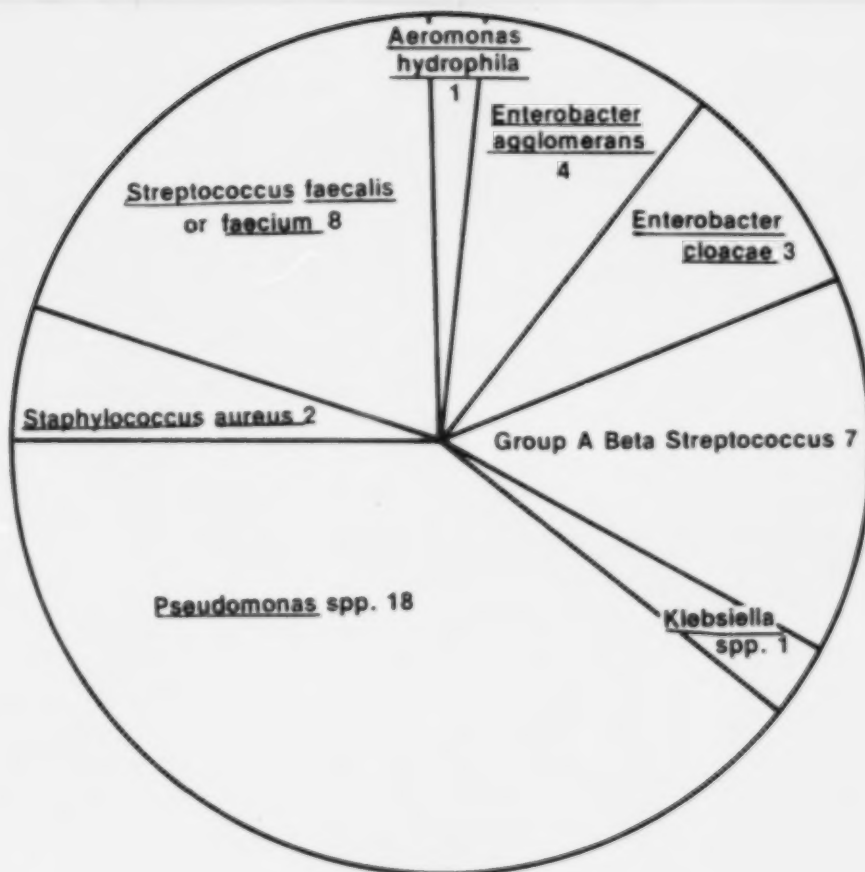


Figure 21. Relative proportions of respirable bacteria identified at other sources^a in 7.46 cubic meters of air.

a. East and West lagoon, farm storage, pump I, pump II, administration stump, road commission, trailer park, grainery, inn, and school.

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

Streptococcus faecalis or faecium. Pseudomonas spp. was relatively more abundant at sampling sites away from the aeration basin and the fields (Figure 21).

DROPPED
HEAD
BEGIN
SECTIONS
HERE

DISCUSSION

Focussing on the results from others, Fair and Wells (90) found a total bacteria count of 787 CFU/m³ at activated sludge aeration tanks and trickling filters using the Wells Air Centrifuge. Using Andersen six-stage sampler at a trickling filter, Albrecht (91) found 5080 CFU/m³ and 2820 CFU/m³ of total bacteria at 15.2 meters downwind. Ladd (92) also using Andersen samplers found total bacteria counts of 2720 CFU/m³ at an activated sludge unit, 1000 to 3040 CFU/m³ at a trickling filter, and 5160 CFU/m³ at a preaeration unit. Carnow et al. (93) found 376 CFU/m³ of total bacteria and 6.87 CFU/m³ of total coliform bacteria using Andersen samplers at an activated sludge basin. Using a high volume sampler at the advanced wastewater treatment plant in Tigard, Oregon, Johnson et al. (94) found 5.8 CFU/m³ of total coliforms, 2.0 CFU/m³ of fecal streptococci, 7 CFU/m³ of Pseudomonas, and 9.1 CFU/m³ of mycobacterium at distances of 30-50 m downwind of an aeration basin. At a spray irrigation site in Pleasanton, California, Johnson et al. (95) using high volume samplers found 7800 CFU/m³ of total bacteria, 43 CFU/m³ of total coliforms, 6.0 CFU/m³ of fecal coliforms, 2.3 CFU/m³ of fecal streptococci, 240 CFU/m³ of Pseudomonas, 280 CFU/m³ of mycobacterium, 4.3 CFU/m³ of Clostridium perfringens, 9.0 CFU/m³ of Streptococcus aerus and 4.1 CFU/m³ Klebsiella at a distance of 20 m from the wet-line edge of spray irrigators. Bausum et al. (96) found 10,000 CFU/m³ of total bacteria and 50 CFU/m³ of Klebsiella using Andersen samplers 46 m downwind at a golf course spray irrigated with wastewater.

Randall and Ledbetter (97) found 42,377 CFU/m³ of total bacteria at an activated sludge site and found that 6% of this total was Klebsiella (or 2542 CFU/m³). Cronholm (98) using 2-stage Anderson samplers found that 18% of the enteric isolates were K. pneumoniae and 42% were K. ozaenae. The maximum total enteric counts averaged about 800 per m³.

The traditional coliform indicators for water pollution are poor indicators of bacterial air contamination because they do not survive as well in air as, for example, the encapsulated Klebsiella. Johnson et al. (89) recommended that fecal streptococci would be a more suitable indicator, while Randall and Ledbetter (97) recommended Klebsiella. It can be calculated that an average adult human breathing 500 ml of air per breath and at a rate of 20 breaths per minute, standing downwind of the aeration basin would inhale about 28 total respirable and 27 gram-negative respirable bacteria per minute. Seventeen percent of the gram-negative bacilli would be Klebsiella spp. The minimum infective dose for man of Klebsiella spp. is not known.

The most significant finding of this work then was the isolation relatively high levels of respirable Klebsiella spp. in the atmosphere downwind of wastewater sources compared to several other recent studies

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA.
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

(93-95). Levels found in this study were comparable to those of Cronholm (98) but lower than those of Ledbetter (97).

DROPPED
HEAD:
BEGIN
SECTIONS
HERE

Klebsiella pneumoniae itself can be found in wet areas around the home such as kitchen sinks and drains (99). It has been found in wooden red wood reservoirs containing finished drinking water and from freshly cut logs (100). Sixty to eighty (60-80) percent of the total coliforms found in raw wastes from pulp mills were identified as Klebsiella (101). The source of Klebsiella at Muskegon is very likely the paper mill located in Muskegon.

It has been found that Klebsiella pneumoniae can survive much longer than the traditional indicator Escherichia coli and even Proteus vulgaris in gas-free mineral water (102). The factors which influenced the multiplication and behavior of p. aeruginosa and K. pneumoniae which were commonly isolated from sink tops were studied in France (103). Klebsiella pneumoniae was more resistant to drying and survived longer than one week. This would mean that Klebsiella pneumoniae survives longer in the environment increasing the probability of infecting the human population. It has also been found that bacteria which are found to be resistant to certain metals, chemicals, or antibiotics are also now likely to be resistant to other metals, chemicals, or antibiotics. The lead-resistant species of Klebsiella isolated from domestic sewage, for example, was found to have simultaneous resistance to other inorganic metals and antibiotics (104). Unfortunately contact with metals, chemicals, and antibiotics and thus resistance to them can be easily achieved by bacteria at a wastewater treatment plant and the more antibiotics the bacteria become resistant to the harder they are for the clinician to treat.

Health Effects to the Immunologically Compromised Patient--

It is important to realize that the health effects of any bacterium are directly related to the patient's immune status. Klebsiella pneumoniae has been found in local infections of patients with both acute leukemia and granulocytopenia (105). Klebsiella oxytoca was isolated from the blood of a leukemia patient in Japan where it was reported that the "typical pathogenicity" of the bacterium was observed (106). Gross et al. (107) performed experiments on animals with complement depletion caused by cobra venom factor. The experiments suggested that hypocomplementemia predisposes to bacterial pneumonia caused by K. pneumoniae and other bacteria. Gross thought that this might explain the unusually high incidence of pulmonary infection which exists in patients who have complement depletion. Harris et al. (108) found that decreasing the amount of available oxygen to the body resulted in impairment of lung clearance of Klebsiella pneumoniae. This was thought to be due to killing by the alveolar macrophage or decreased amount of phagocytosis. Klebsiella has not only been implicated in infection of the lungs, but also the ears, skin, horse's genitals and even gun shot wounds (109). All of these sites are susceptible to exposure by crop spraying of wastewater.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD;
BEGIN
SECTIONS
HERE

Other Health Effects--

Another important point which must be discussed is the possibility of the Klebsiella bacterium being an allergen. An allergen doesn't have to be living, so the bacterial counts might underestimate the amount of allergen. In studying 19 patients with allergic rhinitis and bronchial asthma and looking for allergies to Klebsiella pneumoniae it was found that the bacterial allergy was more important than the bacterial infection (110). Thus there is a need for more studies on the immune status of the susceptible human population in general terms, in terms of allergic response of the bacterium Klebsiella, and in terms of the population's susceptibility towards Klebsiella.

6-1/2"

9-1/8"

BEGIN
LAST LINE
OF TEXT

3/8"

137

PAGE NUMBER

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

SECTION 9

SELECTED ORGANIC CHEMICALS IN AEROSOLS AND WASTEWATER AT A
WASTEWATER SPRAY IRRIGATION SYSTEM

Community and occupational health concerns regarding wastewater treatment facilities have generally focused on aerial dispersion of pathogenic viruses and bacteria and odor discharge from the treatment processes. Recently, accidental or intentional discharges of chemicals from industrial sources have drawn attention to the presence of toxic chemicals in wastewater and their potential release into the atmosphere (64,65). The waste effluents from many industrial installations eventually enter municipal treatment facilities and eventually are discharged to streams, lakes or oceans. These effluents can contain volatile contaminants emanating from raw materials, products, by-products and cleanup operations of manufacturing processes. Regardless of the source, it is highly probable that some quantity of volatile or semi-volatile contaminants will enter the waste stream of many wastewater treatment facilities. The importance of air release of toxic chemicals from wastewater at treatment plants needs evaluation.

The increasing quantity of wastewater sludges being produced and the prohibition of ocean dumping of such sludges have led to renewed interest in wastewater treatment methods that provide reclamation and renovation of wastewater and/ or sludge by land application. One type of wastewater treatment involving land application of wastewater is currently being practiced at the Muskegon, Michigan, Wastewater Management System No. 1 (MWMS). A description of this facility is presented in Section 7 of this report. This system would be predicted to have a high potential for release of volatile chemicals into the atmosphere.

The purpose of this study was to evaluate the release of volatile organic compounds during the treatment of wastewater at the MWMS, to determine if significant exposure of chemicals to the workers at the facility occurred and to determine if significant amounts of chemicals are released to the atmosphere of the nearby community.

Air release of volatile organics from wastewater treatment facilities can occur by three mechanisms: evaporation, air stripping and aerosol formation (111,112,113). The treatment processes at the Muskegon system where air release may occur are aeration, lagoon impoundment and spray irrigation. All three mechanisms contribute to release at the aeration cells where air enters the wastewater by means of mechanical surface aerators. Release from the storage lagoons will occur by evaporation. Aerosol formation and

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

evaporation mechanisms are responsible for air concentration during the spray irrigation operation.

EXPERIMENTAL METHODS

Air release of volatile organic chemicals from wastewater at each treatment process was assessed by concurrently collecting samples of air and wastewater at each treatment site. Wastewater samples were used to determine the amount of specific chemicals potentially available for release into the air. Collection of influent and effluent samples at each treatment process also provided information on the decrease in concentration achieved by the particular treatment. Air samples were collected upwind and downwind of each treatment process. These samples enabled determination of the amount of contaminant in the air that was released from the treatment process.

Sampling and Analysis Procedures

Wastewater grab samples were collected at the influent and effluent of the aeration basins, at one of the irrigation rig pump stations, (influent to irrigation rigs), and from the field drainage ditch. The wastewater samples were collected close to the surface of the wastewater with a galvanized bucket. The samples were immediately transferred into glass bottles (approximately 150 mL) which were filled to minimize the head space. The bottles were sealed with a foil-lined cap, wrapped with aluminum foil, and stored under refrigeration.

The concentrations of selected chlorinated organic contaminants in the wastewater samples were determined by extraction of the water with petroleum ether. A 5 mL aliquot of wastewater was saturated with sodium chloride and 5 mL of petroleum ether was added. The solution was then mixed for 5 minutes on a mechanical shaker. A 5 μ L aliquot of the petroleum ether extract was injected into a gas chromatograph equipped with an electron capture detector (Ni^{63}).

Air samples collected at the aeration basins were obtained either by placing the samplers on the concrete ledges of the basins, which were approximately 1 meter above the water surface and 2 meters from the edge of the water surface, or by attaching the samplers to tripods located approximately 1.5 meters above the water surface and 2 to 3 meters away from the water. The location of air samples collected near the irrigation rigs varied because of the rotation of the rig. The downwind samples were collected 5 to 50 meters from the rigs and 1.5 meters above the ground. Upwind samples were collected 30 to 50 meters from the rigs and 1.5 meters above the ground.

The air samples were collected on adsorbent tubes containing 100 mg of Chromosorb 102 and in most cases charcoal tube samples were also collected. The air was drawn through the sorbent by MSA Model G pumps for high flow rate samples and MDA Accuhaler pumps for low flow rates. The pumps were calibrated before and after each sampling.

END OF
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

The sorbents were desorbed by placing the sorbent in 2 mL of petroleum ether and allowing the mixture to stand for one hour with occasional shaking. A 5 μ L aliquot was then analyzed by gas chromatography with electron capture detector.

RESULTS AND DISCUSSION

Initial air sampling surveys were conducted during April and May 1979. The April survey was limited to monitoring near the aeration basins and outside the Administration Building located on the site. Spray irrigation was not being practiced at that time since the fields were being prepared for planting. The aeration basins and the spray irrigation operations were considered to have the highest potential for aerial release of chemical contaminant from the wastewater. Upwind and downwind samples were obtained at each treatment process in order to evaluate the extent of aerial release of substances from the wastewater. Due to fluctuating wind directions, aerial transport of contaminants may occasionally cause them to reach the upwind sampling locations.

Table 57 presents the results from the initial air monitoring surveys. Three chlorinated organics, chloroform, carbon tetrachloride and tetrachloroethylene, were found in the samples. The downwind air samples collected at the aeration basins showed significant concentrations of these substances. In the May survey samples were obtained downwind of spray irrigation rigs and the storage lagoons. Table 57 shows that only trace amounts of these substances were present in the air at these locations. Wastewater samples were not obtained during these preliminary surveys so comparison of the air concentrations with wastewater levels cannot be made with these samples.

Table 58 and 59 show the results of detailed study of the aerial release of four volatile chlorinated organics from wastewater at the aeration basins. Influent and effluent wastewater samples as well as upwind and downwind air samples were obtained during four sampling periods of one to three days duration each from June through October 1979. The aeration process markedly reduces the wastewater concentrations of chloroform and trichloroethane. Trichloroethylene and tetrachloroethylene do not always appear to be as easily removed. The downwind air concentrations show that significant quantities of these volatile substances are stripped from the wastewater during aeration. The influent and effluent wastewater concentrations represent the daily means during the air sampling period, and it should be recognized that the retention time in the aeration basins is several days. Thus, the difference between influent and effluent concentration may not necessarily represent the actual amount of material stripped, but it at least provides an index of estimation.

Air concentration of the chlorinated organics around the spray irrigation operation are given in Table 60. Relatively low levels of trichloroethane, trichloroethylene and tetrachloroethylene were found. Chloroform was not found in either upwind or downwind air samples. In general, the air concentrations reflect the relatively low concentration of these substances in the

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

3/8"

TABLE 57. INITIAL AIR SAMPLING SURVEYS AT MUSKEGON
COUNTY WASTEWATER MANAGEMENT SYSTEM

Location	Concentration, $\mu\text{g}/\text{m}^3$		
	Chloroform	Carbon tetrachloride	Tetrachloroethylene
April 1979			
Aeration Basin (Upwind)	17	13	53
Aeration Basin (Downwind)	189	1098	252
Outside Admin. Bldg.	6	32	17
May 1979			
Irrigation Rig	--	0.3	1.3
Storage Lagoon	--	ND ^a	3.1
Outside Admin. Bldg.	--	ND	ND
Perimeter of Site	--	0.2	0.5

--Not analyzed.

^aND - None detected, concentrations are less than $0.1 \mu\text{g}/\text{m}^3$.

TABLE 58. DAILY AIR CONCENTRATIONS NEAR AERATION BASINS AND ASSOCIATED WASTEWATER CONCENTRATIONS

	Chloroform			
	Wastewater Conc., ^a μg/L		Air Conc., ^a μg/m ³	
	Influent	Effluent	Upwind	Downwind
6/11/79	892	68	0.5	39 ^b
6/12/79	1180	82	0.5	36 ^b
6/13/79	1520	75	0.5	7 ^b
7/16/79	670	28	0.5	96 ^c
7/17/79	480	18	0.5	202 ^d
7/18/79	800	22	0.5	124 ^d

^aThe values are individual samples or means of 2 to 4 samples.

^bChromosorb 102, high flow, basin-3.

^cCharcoal, low flow, basin-3.

^dCharcoal, low flow, basin-2.

TABLE 59. DAILY AIR CONCENTRATIONS AT AERATION BASINS AND INFLUENT AND EFFLUENT WASTEWATER
CONCENTRATIONS OF THE AERATION BASINS^a

	Trichloroethane						Trichloroethylene						Tetrachloroethylene					
	Wastewater Conc., $\mu\text{g/L}$			Air Conc., ^b $\mu\text{g/m}^3$			Wastewater Conc., $\mu\text{g/L}$			Air Conc., ^b $\mu\text{g/m}^3$			Wastewater Conc., $\mu\text{g/L}$			Air Conc., ^b $\mu\text{g/m}^3$		
	Influ.	Efflu.	Upwind	Downwind	Influ.	Efflu.	Influ.	Efflu.	Upwind	Downwind	Influ.	Efflu.	Influ.	Efflu.	Upwind	Downwind	Influ.	Efflu.
7/16/79	56	ND ^c	0.2	10	1.8	ND	1.8	ND	ND	11.5	3.0	ND	ND	ND	ND	ND	ND	6.8
7/17/79	67	12	4.6	22	69	110	69	110	ND	3	63.3	60.6	ND	60.6	ND	ND	8	8
7/18/79	60	17	3.2	23	118	140	118	140	2.5	73	92.5	88	88	88	9.4	34	34	34
8/20/79	ND	ND	7.3	90	ND	ND	ND	ND	0.9	17	ND	ND	ND	ND	ND	6.5	34	34
8/21/79	72	3.0	7	77	30	12	30	12	0.8	14	25	1.0	25	1.0	0.6	23	23	23
10/17/79	4.4	0.3	ND	17 ^d	7.5	0.7	7.5	0.7	2.4	8.8	7.7	0.4	7.7	0.4	1.8	8.7	8.7	8.7
10/17/79	3.5	0.6	4.7	33	6.7	1.0	6.7	1.0	3.0	40	8.9	1.0	8.9	1.0	8.6	46	46	46

^aThe American Conference of Governmental Industrial Hygienists (ACGIH) has recommended a Threshold Limit Value (TLV) of 1,900 mg/m^3 for trichloroethane, 270 mg/m^3 for trichloroethylene and 670 mg/m^3 for tetrachloroethylene.

^bAir samples were collected using charcoal and Chromosorb 102 sampling tubes at flow rates of 25 to 60 mL/min. The values are means of 2-5 samples.

^cND - designated not detected; concentrations are less than 0.1 $\mu\text{g/m}^3$; wastewater are less than 0.1 $\mu\text{g/L}$.

^dThis value is the mean of 10 samples with a range of 0.2 to 103 $\mu\text{g/m}^3$.

TABLE 60. DAILY CONCENTRATIONS OF SELECTED CHLORINATED ORGANICS NEAR SPRAY IRRIGATION RIGS

Dichloroethane				Trichloroethylene				Tetrachloroethylene			
Wastewater Conc., $\mu\text{g/L}$		Air Conc., $\mu\text{g}/\text{m}^3$		Wastewater Conc., $\mu\text{g/L}$		Air Conc., $\mu\text{g}/\text{m}^3$		Wastewater Conc., $\mu\text{g/L}$		Air Conc., $\mu\text{g}/\text{m}^3$	
N.P. ^a Stat.	Field ^b Efflu.	Upwind	Downwind	N.P. Stat.	Field Efflu.	Upwind	Downwind	N.P. Stat.	Field Efflu.	Upwind	Downwind
7/16/79 Rig 11	1.3	ND	ND	1	ND	ND	ND	ND	ND	ND	ND
7/16/79 Rig 12	1.3	ND	0.2	1	ND	0.4	2.6	ND	ND	1.2	2.1
7/17/79 Rig 11	11	ND	ND	122	33	ND	ND	72	7	ND	ND
7/18/79 Rig 11	5	0.0	1.3	68	79	2.2	9.3	40	43	1.8	8.6
7/18/79 Rig 11	5	0.7	1.3	68	79	0.7	5.0	40	43	0.6	4.6
8/20/79 Rig 12	0.2	1.9	2.3	ND	ND	0.1	0.4	ND	ND	0.3	0.5
8/21/79 Rig 12	3	2.6	2.7	11	25	0.5	0.6	1	26	0.1	0.1

^aSpray rig influent wastewater collected at North Pump Station.

^bField effluent water collected from drainage ditch that collects leachate from irrigated fields for discharge to surface waterways. These samples were collected at the same time as the pumping station samples and therefore represent wastewater sprayed onto the fields prior to the time of collection of the pumping station sample.

ND - designates not detected; air concentrations are less than $0.1 \mu\text{g}/\text{m}^3$; wastewater are less than $0.1 \mu\text{g/L}$.

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
PAGE

ENDED
HERE
BEGIN
SECTION
HERE

water being applied. The highest concentration in the water being spray irrigated was 122 ppb for trichloroethylene. The highest air concentration was $9 \mu\text{g}/\text{m}^3$ for trichloroethylene.

The highest air concentrations were obtained inside the scrubber house at the aeration basins. As shown in Table 61 chloroform was found at a level of $3380 \mu\text{g}/\text{m}^3$ (approximately $3.4 \text{ mg}/\text{m}^3$). The results for air samples obtained at three off-site locations are also shown in Table 61. One of the off-site locations (lakeshore) had measurable amounts of the chlorinated compounds. This sample was collected near the paper mill whose discharge enters MWMS.

Preliminary studies of air levels of chloroform, trichloroethane, trichloroethylene and tetrachloroethylene at other wastewater treatment facilities indicates that the levels found at the aeration basins of the Muskegon Treatment facility are comparable to or in some cases less than air levels found at these other types of wastewater treatment facilities (Tables 62 and 63).

All air concentrations measured were well below recommended occupational limits (38). In the event of accidental discharges of large amounts of these chemicals, areas near the aeration basins, and in particular in the scrubber house, would be expected to experience the highest air concentrations.

TABLE 61. AIR CONCENTRATIONS OF SELECTED CHLORINATED ORGANICS AT VARIOUS LOCATIONS

	Concentration, $\mu\text{g}/\text{m}^3$			
	Chloroform	Trichloro-ethane	Trichloro-ethylene	Tetrachloro-ethylene
Inside Scrubber House	3380	805	306	240
Off-Site Locations;				
Road Commission	ND ^a	ND	ND	ND
Cottage Grove	ND	ND	ND	ND
Lakeshore	24	3.1	1.9	1.2

^aND - None detected, concentrations are less than $0.1 \mu\text{g}/\text{m}^3$.

TABLE 62. AIR SAMPLES COLLECTED AT A 30 MILLION GALLONS PER DAY PRIMARY WASTEWATER TREATMENT PLANT

Location	Concentration, $\mu\text{g}/\text{m}^3$ ^a			
	Chloroform ^b	Trichloro- ^c ethane	Trichloro- ^c ethylene	Tetrachloro- ^c ethylene
Top of Sed. Basin	0.5	1.0	0.3	1.4
Upwind-NE Fence	1.6	0.4	0.1	0.4
Inside Sludge Dewatering Bldg.	1.2	5.3	7.5	12.3
Enclosed Grit Chamber (Detritor Tank)	18	61	10	36
Bar Screen (Wet Well)	53	325	66	145

^aData represent single samples at each location.

^bCollected on charcoal tubes.

^cCollected on Chromosorb 102 tubes at flow rate of 30-50 mL/min.

TABLE 63. AIR CONCENTRATIONS OF SELECTED ORGANICS COLLECTED AT
VARIOUS LOCATIONS AT A 300 MILLION GALLONS PER DAY
ACTIVATED SLUDGE WASTEWATER TREATMENT PLANT

Location	Concentration, $\mu\text{g}/\text{m}^3$ ^a			
	Chloroform ^b	Trichloro- ^c ethane	Trichloro- ^c ethylene	Tetrachloro- ^c ethylene
Enclosed Aerated Grit Chamber	84	20	15	214
Wet Well Pumping Bldg.	47	26	15	139
Secondary Aeration Basin	11	2	1	14
Inside-Sludge Proc. Bldg.	46	2	10	3

^aData represent single samples at each location.

^bCollected on charcoal tubes.

^cCollected on Chromosorb 102 tubes at flow rate of 30-50 mL/min.

SECTION 10

SERO-EPIDEMIOLOGIC SURVEY OF WORKERS AT A WASTEWATER SPRAY IRRIGATION SYSTEM

Spray application, one of the commonly used methods of land application of wastewater, promotes aerosol formation. The viruses present in wastewater may become airborne during spray application and are thus a source of exposure to the workers involved. In order to assess the potential health risks to workers from exposure to viruses, virus antibody levels, isolations and illness rates of the workers at the Muskegon Wastewater Management System, Michigan, were evaluated over a 5-month period and compared with a control group of road commission workers.

METHODS

The populations compared were workers at the Muskegon County Wastewater Management System No. 1 and the Muskegon County Road Commission in Michigan. A description of the spray irrigation treatment system has been presented in Section 7. The Muskegon County Road Commission facilities are located on the north side of Apple Avenue, east of Maple Island Road (see Figure 5).

Serum specimens and throat and rectal swabs were obtained from the study volunteers on a monthly basis between June and October 1979. Illness symptoms experienced during the prior month were obtained during the July-October visits with the study participants. The form used for this purpose is shown in Appendix A.

Viral cultures were inoculated into three types of cell cultures, including primary African green monkey kidney, Vero monkey kidney and WI-38 cells. These were incubated at 35°C, and examined daily for evidence of viral cytopathic effect. Viruses, including those recovered from wastewater, were identified by neutralization with Lim-Benyesh-Melnick antisera pools.

Antibody to enteroviruses was measured by a microneutralization assay. The viruses selected for the survey were poliovirus types 1, 2 and 3, coxsackievirus types B2 and B5, and echovirus types 7 and 11. These viruses were chosen because they were isolated from the study population and wastewater samples, or were the most common enteroviruses in the Muskegon area during the study. Antibody to hepatitis A virus was measured by a competitive radioimmunoassay.

RESULTS

Volunteers Recruited

A total of 35 workers at the wastewater spray irrigation system and 41 at the Road Commission were recruited into the study. The age, race, sex and length of time on the job for these volunteers is summarized in Table 64. The median age of the Road Commission workers is 10 years higher than that of the spray irrigation workers. The difference in number of years on the job reflects the fact that the spray irrigation system began operation in 1973 with the first workers hired in 1972 whereas the Road Commission was established in 1894.

On the basis of interview and job observation data, workers at both the spray irrigation and road commission facilities were divided into three relative exposure categories. The spray irrigation workers in the high exposure category had routine exposure to both wastewater and aerosols. Within this group, there was a subgroup of six past and present nozzle cleaners who were judged to have the highest exposure. Nozzle cleaners are college-age students hired for the summer vacation months for the purpose of regularly cleaning nozzles on the spray irrigation rigs and for performing other tasks. The intermediate exposure spray irrigation system workers had some exposure to either wastewater or aerosols but not to both. The low exposure spray irrigation system workers had no exposure to wastewater and no exposure to aerosols except that which might occur in driving on the roadway leading to the administration building. This roadway passes about 100 meters from several of the irrigation rigs. The road commission workers were divided into two outdoor groups and one indoor group. The age, race, sex and length of time on the job for study participants, divided according to exposure category, is summarized in Table 65. The nozzle cleaners were the youngest group, with a median age of 21 years.

Reported Illnesses

Illness symptoms reported during the monthly interview were tabulated in the categories: respiratory, gastrointestinal, respiratory plus gastrointestinal and other. A comparison of the number of illnesses by illness type for the spray irrigation and road commission workers, by total group and by exposure category (Table 66) revealed no differences. The overall illness rate/worker month was practically identical for the spray irrigation workers and the road commission workers, 0.54 and 0.58, respectively.

Virus Isolations

Coxsackie B5 virus was isolated from both the throat and rectal swabs collected in August from one road commission participant. An extensive interview with this participant failed to detect any evidence of symptoms. No other isolates were obtained from throat and rectal swabs during the study.

TABLE 64. AGE, RACE, SEX AND LENGTH OF TIME ON THE JOB FOR SPRAY IRRIGATION SYSTEM AND ROAD COMMISSION STUDY PARTICIPANTS

	Spray Irrigation System	Road Commission
Total Number*	35(2)	41(7)
Age, years		
Median	28	38
Range	19-61	25-69
Race		
White*	33(2)	35(5)
Non-white*	2	6(2)
Length of time on job, years		
Range	<1-7	<1-25

*Numbers of which are female appears in parenthesis.

TABLE 65. AGE, RACE, SEX AND LENGTH OF TIME ON THE JOB FOR STUDY PARTICIPANTS DIVIDED ACCORDING TO EXPOSURE CATEGORY

	Spray Irrigation System		Road Commission Exposure Category			
	High*	Int.	Low	Out-side 1**	Out-side 2**	In side
Total Number***	12	10	13(2)	18	5	18
Age, years						
Median	27	32	27	34	36	40
Range	19-61	24-49	22-61	29-62	28-59	25-69
Race						
White***	11	9	13(2)	15	4	16(5)
Non-White***	1	1	0	3	1	2(2)
Length of time on job, years	<1-7	<1-7	<1-7	<1-25	5-17	<1-24

*For the six nozzle cleaners, the median age was 21 years with a range of 19-28; five were white and the length of time on the job was less than one year for five and 2 years for the one non-white worker.

**Outside 1 workers have soil exposure, outside 2 do not.

***Numbers of which are female appears in parenthesis.

TABLE 66. COMPARISON OF ILLNESS FOR SPRAY IRRIGATION AND ROAD
COMMISSION WORKERS BY ILLNESS TYPE, WORKER GROUP,
AND EXPOSURE CATEGORIES

Worker Group	No. Worker Months	No. Illnesses				Total
		Respira- tory	Gastroin- testinal	Resp. & Gastro.	Other	
Spray Irrigation	112	33	18	1	9	61
High Exposure	35	10	5	0	4	19
Int. Exposure	39	11	5	0	4	20
Low Exposure	38	12	8	1	1	22
Road Commission	130	52	12	3	8	75
Outdoor-soil contact	54	23	6	0	4	33
Outdoor-Other	17	10	0	0	1	11
Indoor	59	19	6	3	3	31

Fourteen viral isolates picked from a sample of the July 17, 1979 raw influent to the aeration basins were identified as follows: two poliovirus III, nine coxsackievirus B2 and three echovirus 7.

Virus Serology

A comparison of the prevalence of antibody for those viruses tested was made between the spray irrigation and road commission workers for that month for which the most persons were active in the study, July. The results of these comparisons are presented in Table 67. Only for Coxsackie B5 was the difference significant with the spray irrigation worker group having a higher proportion of titers ≥ 2 .

Increases of fourfold or greater in titer level of a particular antibody (2 or more serum dilutions) indicate infection in the individual involved. In order to evaluate differences in the number of fourfold or greater titer level rises results from the first and last serum from each participant were compared. Comparisons were made for all spray irrigation and road commission workers. Results, presented in Table 68, reveal no differences for any of the viruses for either of the groups compared.

The titer level of coxsackie B5 antibody of the workers from whom coxsackie B5 virus was isolated increased from ≤ 2 at the time of the isolation to ≥ 128 one month later.

Anti-HAV sera from 8 of the 35 spray irrigation workers tested positive while for the road commission workers the proportion was 17 of 41. A comparison of the results divided according to age, < 30 years, ≥ 30 years, < 40 years and ≥ 40 years (Table 69) indicate no significant differences (chi-square, $X^2 \geq 0.10$).

In an effort to further evaluate the effect of age on antibody titer levels a group of 31 spray irrigation and road commission workers were selected. This group is composed of 13 low or non-exposed spray irrigation system workers and the 18 indoor road commission workers. Together their ages ranged from 22 to 69. Using Pearson correlation coefficients as well as X^2 and Fisher's exact test, age was found to be directly (and categorically) related to the presence of antibodies to Coxsackie B5 as well as Hepatitis A. As age increases the probability of finding a "positive" (≥ 2) titer level increases. Exemplary X^2 tables are:

		Titer Levels to Coxsackie B5		Test for Antibodies to Hepatitis A	
		< 2	≥ 2	Negative	Positive
Age	< 30	9	1	10	0
	≥ 30	8	13	10	11

$p < 0.01$ $\leftarrow$ by Fisher's exact test $\rightarrow p < 0.005$

TABLE 67. COMPARISON OF PREVALENCE OF ANTIBODY AT TITER LEVEL 2 OR GREATER FOR ALL SPRAY IRRIGATION AND ROAD COMMISSION WORKERS DURING THE MONTH OF JULY

		Titers		
		<2	≥2	
		Negative	Positive	
Echo 11	Spray Irrigation	11	21	p > .10
	Road Commission	11	25	
Coxsackie B5	Spray Irrigation	13	19	p = .03
	Road Commission	24	12	
Polio I	Spray Irrigation	4	28	p > .10
	Road Commission	4	32	
Polio II	Spray Irrigation	0	32	p > .10
	Road Commission	1	35	
Polio III	Spray Irrigation	1	31	p > .10
	Road Commission	3	33	
Coxsackie B2	Spray Irrigation	11	21	p > .10
	Road Commission	13	23	
Echo 7	Spray Irrigation	27	5	p > .10
	Road Commission	25	11	

p by χ^2 test or Fisher's exact test wherever appropriate.

TABLE 68. COMPARISON OF NUMBER OF FOURFOLD OR GREATER TITER LEVEL
INCREASES IN SPRAY IRRIGATION AND ROAD COMMISSION
WORKERS USING RESULTS FROM FIRST AND LAST SERUM
COLLECTED FROM EACH PARTICIPANT

Virus	Group	No. Participants	No. Fourfold and Greater Titer Level Increases
Polio I	Spray Irrigation	33	5
	Road Commission	40	5
Polio II	Spray Irrigation	33	2
	Road Commission	40	2
Polio III	Spray Irrigation	33	3
	Road Commission	40	1
Coxsackie B2	Spray Irrigation	33	2
	Road Commission	40	5
Coxsackie B5	Spray Irrigation	33	3
	Road Commission	40	1
Echo 7	Spray Irrigation	33	1
	Road Commission	40	1
Echo 11	Spray Irrigation	33	1
	Road Commission	40	0
Total	Spray Irrigation	33	17
	Road Commission	40	15

TABLE 69. COMPARISON OF HEPATITIS A ANTIBODY TESTS FOR SPRAY
IRRIGATION AND ROAD COMMISSION WORKERS
AT BOTH AGE 30 AND AGE 40

Anti-HAV			
Age <30		Age <40	
	Negative	Negative	Positive
Spray Irrigation	18	23	4
Road Commission	4	19	7

Anti-HAV			
Age ≥30		Age ≥40	
	Negative	Negative	Positive
Spray Irrigation	9	4	4
Road Commission	20	5	10

Using age 30 for partitioning seems crucial in establishing the age difference for Cocksackie B5 as not statistically significant ($p = 0.10$) difference manifests itself if we use age 40. For Hepatitis, however, the significant difference is unaffected by a choice of age 30 or 40. Although no significant age effect was statistically discernable for the other viruses studied (Echoviruses 7 and 11, Cocksackie B2, and Polios I, II and III) they may, in fact, exist. For our small number of individuals they did not reveal themselves.

Antibody prevalence comparison between the high exposure spray irrigation system workers and the indoor road commission workers (Table 70) revealed no significant differences for the month in which the highest number of high exposure workers were tested (June). In order to evaluate differences in the number of fourfold or greater titer level rises, results from the first and last serum were compared for the high exposure spray irrigation workers and the indoor road commission workers. Results, presented in Table 71 reveal no differences for any of the viruses for either of the groups compared.

Included in the group of 12 participants in the high exposure spray irrigation workers is a sub-group of nozzle cleaners who are thought to have the highest exposure. Nozzle cleaners are part-time employees hired for the summer and are generally college students. Five current and one former nozzle cleaners were participants in the study. Four of the six had previously spent at least one summer as a nozzle cleaner, the other two had been on the job for a month before the collection of the first specimen for the study. To obtain statistically significant differences (at say $p < 0.05$) for only 6 individuals requires a near total polarity in data. This did occur in the case of the virus Cocksackie B5. All 6 individuals had positive (≥ 2) results for all bloods drawn with the minimum titer being 16. Since it has previously been shown that age is directly related to the probability of an individual having a positive antibody titer to coxsackie B5 and the nozzle cleaners are aged 19-28 (all under 30), a positive titer would be expected to occur by chance in only one out of ten persons.

Because one nozzle cleaner quit work after only 1 month of the study and other summer nozzle cleaners were back in school by September and October the "optimum" month for comparison was June since that was the only month when all were in the study. A comparison was made of June Cocksackie B5 titer levels between the nozzle cleaners and various other worker groups: all road commission workers, outdoor road commission workers with soil exposure, indoor road commission workers, all spray irrigation workers, other high exposure spray irrigation workers, other high exposure spray irrigation workers and intermediate exposure spray irrigation workers, and low exposure spray irrigation workers. For all these comparisons the significance levels, determined by Fisher's exact test were: ≤ 0.05 (Table 72). All 6 of the past and present nozzle cleaners showed negative results for anti-HAV of the remaining 6 high exposure spray irrigation workers, only 2 individuals were positive.

TABLE 70. COMPARISON OF PREVALENCE OF ANTIBODY AT TITER LEVEL 2 OR GREATER FOR HIGH EXPOSURE SPRAY IRRIGATION WORKERS AND INDOOR ROAD COMMISSION WORKERS DURING MONTH OF JUNE

		Titers		
		<2	≥2	
Echo 11	Spray Irrigation High Exposure	2	10	$p > 0.10$
	Road Commission Indoor Workers	5	9	
Coxsackie B5	Spray Irrigation High Exposure	4	8	$p > 0.10$
	Road Commission Indoor Workers	8	6	
Polio I	Spray Irrigation High Exposure	3	9	$p > 0.10$
	Road Commission Indoor Workers	2	12	
Polio II	Spray Irrigation High Exposure	0	12	$p > 0.10$
	Road Commission Indoor Workers	1	13	
Polio III	Spray Irrigation High Exposure	1	11	$p > 0.10$
	Road Commission Indoor Workers	1	12	
Coxsackie B2	Spray Irrigation High Exposure	3	9	$p > 0.10$
	Road Commission Indoor Workers	8	6	
Echo 7	Spray Irrigation High Exposure	11	1	$p > 0.10$
	Road Commission Indoor Workers	10	4	

p by Fisher's exact test.

TABLE 71. COMPARISON OF NUMBER OF FOURFOLD OR GREATER TITER LEVEL INCREASES IN HIGH EXPOSURE SPRAY IRRIGATION WORKERS AND INDOOR ROAD COMMISSION WORKERS USING RESULTS FROM FIRST AND LAST SERUM COLLECTED FROM EACH PARTICIPANT

Virus	Group	No. Participants	No. Fourfold and Greater Titer Level Increases
Polio I	High Exposure Spray Irrigation	11	2
	Indoor Road Commission	17	2
Polio II	High Exposure Spray Irrigation	11	0
	Indoor Road Commission	17	0
Polio III	High Exposure Spray Irrigation	11	1
	Indoor Road Commission	17	1
Coxsackie B2	High Exposure Spray Irrigation	11	2
	Indoor Road Commission	17	2
Coxsackie B5	High Exposure Spray Irrigation	11	1
	Indoor Road Commission	17	1
Echo 7	High Exposure Spray Irrigation	11	0
	Indoor Road Commission	17	1
Echo 11	High Exposure Spray Irrigation	11	1
	Indoor Road Commission	17	0
Cumulative	High Exposure Spray Irrigation	11	7
	Indoor Road Commission	17	7

TABLE 72. COMPARISON OF JUNE COXSACKIE B5 TITER LEVELS FOR NOZZLE CLEANERS WITH THOSE OF VARIOUS OTHER GROUPS OF STUDY PARTICIPANTS

	June Coxsackie B5 Titers			June Coxsackie B5 Titers	
	<2	≥2		<2	≥2
	Negative	Positive		Negative	Positive
Nozzle cleaners	0	6	Nozzle cleaners	0	6
Outdoor Road Commission Workers Workers-soil Contact	9	6	Other Spray Irrigation High Exposure	4	2
p = 0.017			p = 0.030		
Nozzle cleaners	0	6	Nozzle cleaners	0	6
Road Commission Indoor Workers	8	6	Spray Irrigation Low Exposure	7	2
p = 0.024			p = 0.006		
Nozzle cleaners	0	6	Nozzle cleaners	0	6
All Road Com- mission	19	15	Spray Irrigation Other High Ex- posure and Int. Exposure	9	6
p = 0.014			p = 0.017		
Nozzle cleaners	0	6	Nozzle cleaners	0	6
All Other Spray Irrigation Workers	16	8	p = 0.005		

Critical levels (p) are determined by Fisher's exact test.

It is apparent that further investigation is warranted in the case of the nozzle cleaner category of workers. These individuals are reported to become frequently soaked when back pressure remains in the nozzles. It is also likely that infection with the virus may occur symptom-free, as was the case with a road commission worker whose titer level increased from ≤ 2 to > 128 in one month from August to September with confirming throat and rectal swab tests but with no noticeable symptoms.

Summary

No differences in illness and virus isolation rates were detected between the 35 spray irrigation workers and the 41 road commission workers. The only virus isolated by routine screening was Coxsackie B5 which was identified in both the throat and rectal swab of an asymptomatic road commission worker whose titer rose from less than 2 to greater than or equal to 128. The spray irrigation workers had elevated levels of antibody to coxsackvirus B5 compared to the control group of road commission workers. Levels of antibody to Coxsackie B5 in a group of six present and former nozzle cleaners were significantly higher than for the other spray irrigation workers, the road commission workers or for several subgroups based on relative exposure. This difference was not due to age differences between the groups, because although the prevalence of the virus tended to increase with age, the spray irrigation workers were, on average, younger than the road commission workers. No differences were detected in levels of antibody to Poliovirus 1, 2 and 3, Coxsackie B2, Echo 7 and Echo 11. Presence of Hepatitis A virus was found to be positively correlated with age, as has been found by others (2,114,115) but there were no differences between groups when corrected for age.

SECTION 11

EFFECTS OF SEWAGE TREATMENT PLANT SLUDGE ON THE STRUCTURE
AND FUNCTION OF TWO GRASSLAND ECOSYSTEMS

Numerous studies have been concerned with the effects of sludge on the cellular (116), histological (117), and organismic (118) levels of biological organization. Most of these studies have focused upon individual plant or animal species or their respective component parts (e.g., concentration of heavy metals within various tissues and organs or effects of certain substances upon individual growth and/or reproductive rates). However, since all species including man, function within natural ecological systems (ecosystems), it is unfortunate that the higher levels of biological organization (i.e., the population, community, and ecosystem levels of integration) have received little attention.

Odum, Barrett, and Pulliam (119) have pointed out that problems often arise when stressors are tested at one level (e.g., individual) and then used without further study at another level (e.g., ecosystem). It is also clear that only by considering the whole ecosystem, as outlined in this report, will man be better able to predict the long-range results of environmental contamination (120). Further, Barrett, Van Dyne, and Odum (120) have outlined specific guidelines for testing stressors at the ecosystem level. We believe "field ecosystem testing" is not only feasible but may also be much less expensive and more realistic than laboratory tests of each species or population separately. It is an ecological axiom that the sum of the parts does not equal the whole (122).

Woodwell (123) challenged engineers and planners to convert cities from open systems which take in fresh water and excrete wastewater to closed systems which reuse their water resources. A possible means of accomplishing this goal is to filter sewage wastes through managed ecosystems. Effective planning should locate such systems in the vicinities of municipalities according to the compartmentalization concept of Odum (124). Indeed, the feasibility of this approach regarding forest and cropland ecosystems has already been tested (125). The "best compartments," (i.e., the most efficient ecosystems) may be an intermediate (natural) old-field plant community in the 3-10 year of old-field succession which would naturally decompose and assimilate the greatest amount of sludge on a per-unit (ha) basis. The reasons for this are related to Table 73. Old-field communities are highly productive (126), exhibit closed inorganic nutrient cycles (127), make effective use of intrabiotic detritus (128), and are considered to be ecologically stable (129). Such early developmental communities could also handle additional nutrient input (extrabiotic input) and still maintain the

TABLE 73. ATTRIBUTES OF THREE STAGES OF ECOSYSTEM DEVELOPMENT
RATED ACCORDING TO THEIR EXPECTED CAPACITY FOR PROCESSING
SEWAGE SLUDGE (Adapted From Odum, 1969) (124)

Ecosystem Attributes	Young (Wheat) Agricultural Stage	Intermediate* (Old-field) Stage	Mature (Forest) Stage
<u>Community energetics</u>			
1. Gross production/standing crop biomass	High	High	Low
2. Net community production	High	High	Low
<u>Community structure</u>			
3. Inorganic nutrients	Extrabiotic	Intrabiotic	Intrabiotic
<u>Life history</u>			
4. Life cycles	Simple	Intermediate	Complex
<u>Nutrient cycling</u>			
5. Mineral cycles	Open	Closed	Closed
6. Nutrient exchange rate	Rapid	Rapid	Slow
7. Role of detritus	Unimportant	Important	Important
<u>Overall homeostasis</u>			
8. Stability	Poor	Good	Good

*Proposed most efficient ecosystem for sewage disposal.

above-mentioned characteristics. In addition, the energy subsidies which operate or drive these systems should be much less as compared to either agricultural or forested systems which frequently require human (energy intensive) management. The producers (plants) are naturally seeded and maintained and the primary consumers, dominated by arthropods, are natural grazers containing high protein content for higher trophic levels. By introducing meadow voles (*Microtus pennsylvanicus*) into these communities the rate of nutrient recycling on a low energy basis would be greatly increased. The voles are effective plant consumers (30) and would also be important warm-blooded monitors concerning growth, reproduction, and population dynamics change.

The objectives of this study were as follows: (a) to measure and evaluate the effects of chronic sludge application on several different levels of biological organization, (b) to compare two different community-types (monoculture vs. old-field) treated in an identical manner, (c) to analyze different structural (e.g., biomass and species diversity) and functional (e.g., energy flow and community resilience) ecosystem parameters--measurements which may be indicative of total system response, (d) to evaluate sludge effects (via ecosystem food chains) on a natural small mammal population (*Microtus pennsylvanicus*) functioning within these community-types, and (e) to develop a feasible ecosystem approach, including the identification of "white mice" field parameters, for testing stressors (e.g., sludge) on intact ecosystems.

METHODS

Land Treatment

The study made use of landspreading techniques to examine the effects of chronic dosages of sludge to compare an old-field plant community with an agricultural (wheat) field community. The experimental system consisted of sixteen one-tenth hectare enclosures of galvanized steel located at the Ecology Research Center at Miami University. The individual plots were randomly selected for treatment with sludge (S), fertilizer (F) or were used as controls (C) as indicated in Figure 22. The sludge used was Milorganite, an anaerobically-digested, heat-dried municipal sludge commercially marketed by the City of Milwaukee, Wisconsin. The sludge was applied to each of a total of six plots at the rate of 181 Kg per month during May-September in 1978 and 1979. Its elemental composition was 6-2-0 (N-P-K).

Commercial fertilizer of composition (34.2-11.3-0) was applied at the rate of 32 Kg per plot per month to provide equivalent added amounts of nitrogen (N) and phosphorous (P) as received by the sludge amended plots. Eight of the plots were planted in Kentucky Bluegrass (*Poa sp*) in 1974 and were in the fourth year of old-field succession in 1978. The other eight were planted in winter wheat in fall of 1977. The wheat matured in July 1978 was not harvested, and was left fallow thereafter. The wheat field had previously been treated as follows:

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
PAGE
AREA

SHOULDER
HEAD
RIGHT
MARGINS
HERE

C	F	S	C
S	F	S	F

OLD FIELD

S	C	F	F
S	C	S	F

WHEAT FIELD

S: Sludge

F: Fertilizer

C: Control

Figure 22 Schematic arrangement of individual study plots.

20 October 1977--plowed and disked;

24 October 1977--fertilized with 300 lbs/acre (336 Kg/ha) commercial fertilizer (12-12-12) and seeded with winter wheat (*Triticum* sp.).

DROPPED
HEAD
BEGIN
SECTION
HERE

A modified garden manure-spreader was used to dispense the dried sludge and fertilizer.

Contamination of control plots by sludge from adjacent experimental plots was minimal and was restricted to the lower soil horizons. Steel enclosure walls surrounding the plots extended 20 inches (60 cm) below the ground surface, thus restricting any lateral flow of nutrients. The only possible source of contamination was through deep root extraction of nutrients leached to lower soil layers.

Sludge and Fertilizer Analyses

Composite samples were made for each month of sludge and/or fertilizer application for 1978 and 1979. Five gram portions from subsamples of the same month were combined and shaken vigorously (six plots, two bags per plot). This composite, which was felt to be representative of the month was then analyzed for Pb, Cd, Cu and Zn in triplicate using 1 gm samples. There were no sludge samples received for the months of June 1979, and there was only an annual composite fertilizer sample for 1979. In this latter case six replicate 1 gm samples were taken.

Sample preparation for analysis by flame atomic absorption spectroscopy involved the use of a wet-ash technique using nitric (HNO_3) and perchloric (HClO_4) acids. Five mL of distilled concentrated nitric acid was added to the sample and the sample was heated gently to dryness. After cooling 10 mL HNO_3 and 10 mL HClO_4 were added. The samples were then heated to fumes of HClO_4 . After cooling, the samples were retreated with additional 5 mL HNO_3 . The samples were again heated and this time allowed to go to dryness. Additional treatment was felt necessary, especially for the sludge samples; consequently, 5 mL portions of each acid were added, and the samples were reheated strongly to dryness. Final dissolution consisted of adding 10 mL of acid solution 5% (v/v) nitric acid and 5% (v/v) hydrochloric acid in demineralized H_2O , concentrating to approximately 2 mL, transferring to 10 mL graduated cylinder, making fresh up to the original volume, and shaking vigorously. The undissolved material was allowed to settle out prior to analysis. Reagent controls were also carried with the samples.

Ecological Methods

Five breeding pairs of meadow voles (*Microtus pennsylvanicus*) were introduced into each of the 16 quarter-acre plots during June of 1978 and 1979. The total of 320 voles was captured from wild (untreated) populations in nearby hayfields.

The study focused on three aspects of the grassland ecosystems which may be affected by sludge deposition: structure and energetics of the total

ecosystems, population dynamics of small mammals, and population dynamics of arthropods.

Comparison between control and experimental plots in each grassland type provided information regarding the effects of sludge on the ecosystem as a whole. Comparison of the experimental plots between the two grassland types provided information as to which ecosystem is better able to process sludge most efficiently.

The effects of sludge on vegetative community structure was considered by comparison of species diversity between control and experimental plots. Productivity was measured by the short term harvest method. Diversity was determined by the number of species per 0.25 m^{-2} , Margalef's richness index, Shannon-Weaver's function, and Pielou's evenness index.

Vole population dynamics in the control and experimental systems were compared on the basis of age structure, turn-over rate, population growth rate, and carrying capacity. Vole population densities were related to vegetation nutrient content in a test of Pitelka and Shultz (131) theories of nutrient-recovery and nutrient cycling effects.

Arthropod response to treatment was evaluated for all major orders in terms of density and biomass. One order (Homoptera) was analyzed in detail regarding species richness and apportionment.

Histopathology

During November of 1978 and 1979 the voles were caught in the morning, numbered, and delivered to the Pathology Section in the Department of Environmental Health at the University of Cincinnati for sacrifice and autopsy. Each day the animals were randomly selected from different fields. A complete autopsy was performed on each vole. Tissues were taken from each organ and fixed in neutral formalin. Histopathology was performed on liver, lung, kidneys, and testes or ovaries. The remaining organs were stored in the fixative in case more extensive examinations are desired.

The tissues were embedded in paraffin, cut at 6 μm thickness, and stained with hematoxylin and eosin. They were histologically examined without knowledge of the treatment. The findings were recorded on the autopsy records. After completion, the code of the different groups was received and the pathology was tabulated.

RESULTS

Metal Content of Sludge and Fertilizer

The results of the analysis of the heat-dried sludge (Milorganite) and the commercial fertilizer for lead, cadmium, copper and zinc are presented in Tables 74 and 75, respectively. For each metal the concentration in the sludge was higher than in the fertilizer. The ratio of the concentration in the sludge to the concentration in the fertilizer in 1978 was 88 for Pb, 30

TABLE 74. METAL CONTENT OF SLUDGE, 1978 AND 1979

	1978			
	Concentration, $\mu\text{g/g}$, mean $\pm$ S.D. ^a			
	Pb	Cd	Cu	Zn
May	448 $\pm$ 10	60 $\pm$ 1	341 $\pm$ 4	1012 $\pm$ 20
June	487 $\pm$ 4	59 $\pm$ 1	325 $\pm$ 5	1088 $\pm$ 12
July	488 $\pm$ 13	60 $\pm$ 2	308 $\pm$ 7	1162 $\pm$ 80
August	476 $\pm$ 10	59 $\pm$ 1	313 $\pm$ 6	1075 $\pm$ 13
September	467 $\pm$ 5	60 $\pm$ 1	315 $\pm$ 4	1062 $\pm$ 93
May-September	473 $\pm$ 17	59 $\pm$ 1	320 $\pm$ 13	1081 $\pm$ 67

	1979			
	Concentration, $\mu\text{g/g}$, mean $\pm$ S.D. ^a			
	Pb	Cd	Cu	Zn
May	454 $\pm$ 14	60 $\pm$ 1	341 $\pm$ 2	1007 $\pm$ 28
June	No samples	No samples	No samples	No samples
July	449 $\pm$ 43	57 $\pm$ 5	313 $\pm$ 38	926 $\pm$ 144
August	467 $\pm$ 18	57 $\pm$ 3	330 $\pm$ 8	959 $\pm$ 22
September	466 $\pm$ 28	57 $\pm$ 2	331 $\pm$ 16	978 $\pm$ 38
May-September	459 $\pm$ 25	58 $\pm$ 3	329 $\pm$ 21	968 $\pm$ 72

^aThe values represent triplicate determinations.

TABLE 75. METAL CONTENT OF FERTILIZER, 1978 AND 1979

1978 Concentration, $\mu\text{g/g}$, Mean $\pm$ S.D.				
	Pb	Cd	Cu	Zn
<u>1978^a</u>				
May	1.8 $\pm$ 0.2	1.6 $\pm$ 0.1	1.4 $\pm$ 0.0	20.8 $\pm$ 0.4
June	5.7 $\pm$ 7.0	2.2 $\pm$ 0.2	1.5 $\pm$ 0.1	19.5 $\pm$ 0.7
July	3.0 $\pm$ 0.8	2.0 $\pm$ 0.1	2.6 $\pm$ 0.7	26.8 $\pm$ 1.6
August	4.9 $\pm$ 6.8	2.3 $\pm$ 0.8	2.2 $\pm$ 0.4	24.5 $\pm$ 6.4
September	10.3 $\pm$ 15	1.7 $\pm$ 0.1	1.4 $\pm$ 0.2	16.7 $\pm$ 0.4
May-September	5.4 $\pm$ 7.6	2.0 $\pm$ 0.4	1.9 $\pm$ 0.6	21.8 $\pm$ 4.6
<u>1979^b</u>				
Annual Composite	12.4 $\pm$ 12.0	1.6 $\pm$ 0.4	1.6 $\pm$ 0.4	16.4 $\pm$ 4.0

^aTriplicate determinations.^bSix replicate determinations.

for Cd, 168 for Cu and 50 for Zn; for 1979 the ratios were 37, 36, 205 and 59, respectively. Since the rate of sludge application, by weight, was 5.6 times that of the fertilizer, appreciably more of these metals were applied to the sludge-treated plots than was indicated by the concentration ratios alone as shown in Table 76.

Primary Productivity and Diversity

Shannon-Weaver's function and evenness in the annual field were significantly lower in the fertilizer than in the sludge treatments (Table 77 and Figure 23) (132,133). No significant differences due to treatment were found in species richness in the annual field or in any diversity index in the perennial field (Table 77 and Figure 24).

There was a significant increase in productivity in both fields in the fertilizer and sludge treatments, however the increase was larger and occurred earlier in the fertilizer treatments (Tables 78 to 81 and Figures 25 and 26). Estimates of net primary productivity in the annual field were $1690 \text{ g}^{-2} \text{ yr}^{-1} + 84 \text{ S.E.}$ in the fertilizer treatment, $1240 \text{ g}^{-2} \text{ yr}^{-1} + 58 \text{ S.E.}$ in the sludge treatment, and $772 \text{ g}^{-2} \text{ yr}^{-1} + 3 \text{ S.E.}$ in the control. Estimates of net primary productivity in the perennial field were $1110 \text{ g}^{-2} \text{ yr}^{-1} + 39 \text{ S.E.}$ in the fertilizer treatment, $684 \text{ g}^{-2} \text{ yr}^{-1} + 69 \text{ S.E.}$ in the sludge treatment, and $424 \text{ g}^{-2} \text{ yr}^{-1} + 37 \text{ S.E.}$ in the control. It appears that the treatment effect on plant community structure was greater in the annual field than in the perennial field, suggesting the perennial community is more stable than the annual field and better suited for application of sludge than the annual field.

Arthropods

Arthropod density and biomass increased in both communities following treatment with fertilizer or sludge (134). The Coleoptera, Diptera, Hemiptera and Homoptera depicted total density and biomass arthropod response to nutrient enrichment in both communities. Significant density differences occurred later in the growing season in the old-field plots as compared to the agricultural plots; the agricultural plots exhibited greater resilience to nutrient perturbation whereas the old-field plots exhibited greater resistance.

Diptera, Hemiptera, and Homoptera in the old-field community exhibited significant increases in biomass as a result of nutrient enrichment. All orders except the Homoptera and Araneida in the agricultural community showed significant increases in biomass as a result of nutrient enrichment. Homoptera and Araneida densities were significantly greater in the old-field plots whereas Hemiptera densities were greater in the agricultural plots. Also, all orders except the Hemiptera and Araneida showed greater biomass values in the old-field as compared to the agricultural community.

The Homoptera, a dominant group of insect herbivores, was used for an analysis of species/unit area, species richness ($D = S - 1/\ln N$) and species apportionment ($e = H'/\ln S$). Fertilizer and sludge plots exhibited consistently higher species richness values than did the control plots in both

TABLE 76. TOTAL AMOUNTS OF THE HEAVY METALS APPLIED TO THE SLUDGE AND FERTILIZER TREATED PLOTS IN 1978 AND 1979 (GRAMS)

Metal	Sludge		Fertilizer		Sludge/Fertilizer 1978	Sludge/Fertilizer 1979
	1978	1979	1978	1979		
Lead	2568.39	2492.37	5.18	11.9	495	209
Cadmium	320.37	314.94	1.92	1.53	167	205
Copper	1737.60	1786.5	1.82	1.53	953	1163
Zinc	5869.83	5256.24	20.93	15.74	280	334

TABLE 77. SHANNON WEAVER'S DIVERSITY INDEX, MARGELEF'S RICHNESS INDEX, AND PIELOU'S EVENNESS INDEX IN THE ANNUAL AND PERENNIAL FIELD BASED ON PEAK BIOMASS OF EACH SPECIES

	Shannon Weaver's Index	Margelef's richness	Pielou's evenness
<u>Annual Field</u>			
Fertilizer	1.29±0.016	3.19±0.239	0.40±0.006
Sludge	1.61±0.237	3.04±0.394	0.52±0.055
Control	1.59±0.080	2.93±0.56	0.51±0.012
<u>Perennial Field</u>			
Fertilizer	1.66±0.080	1.87±0.217	0.54±0.081
Sludge	1.61±0.097	2.34±0.681	0.59±0.075
Control	1.63±0.125	1.82±0.202	0.67±0.060

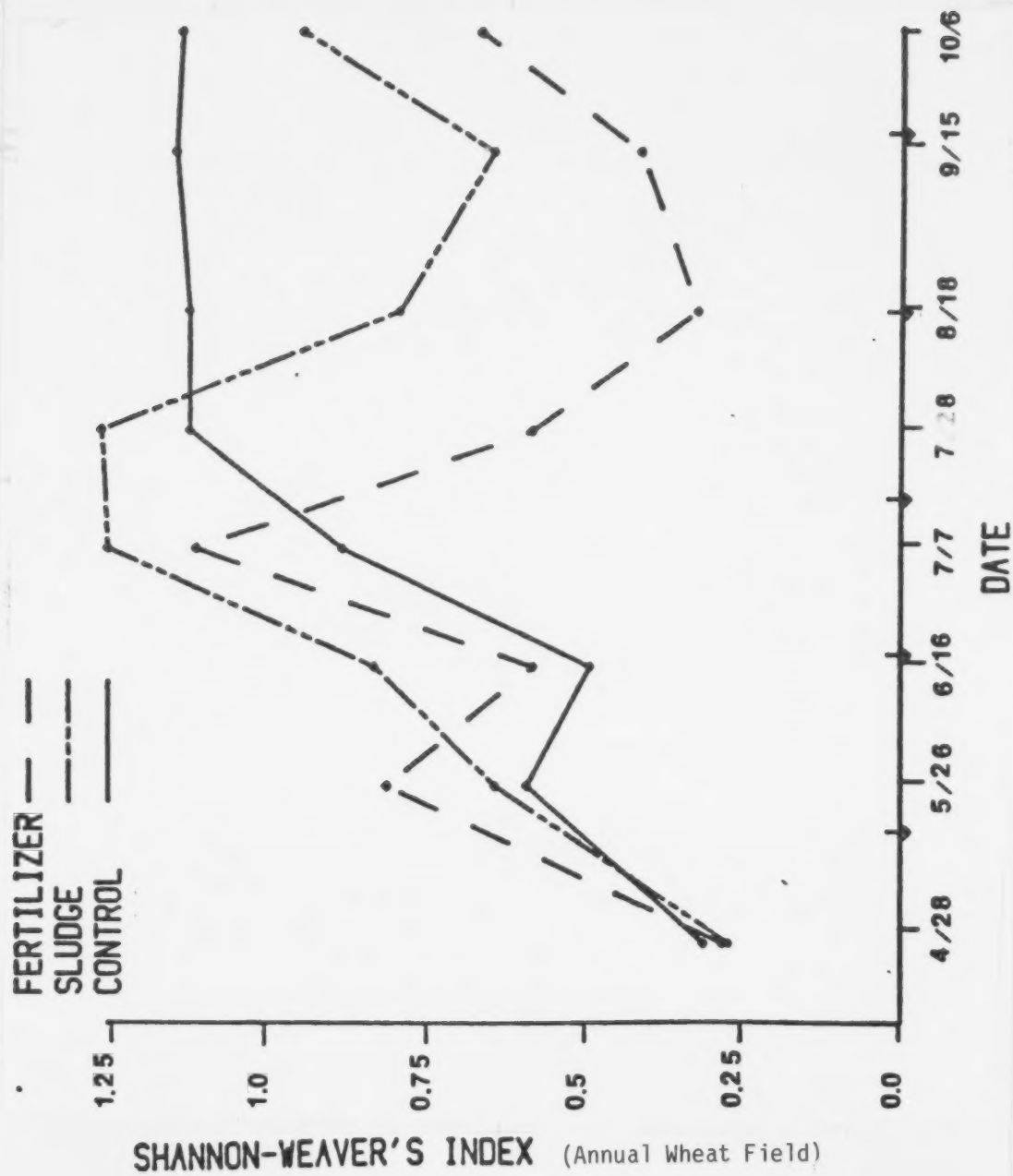


Figure 23. Shannon-Weaver's Index in the annual wheat field.

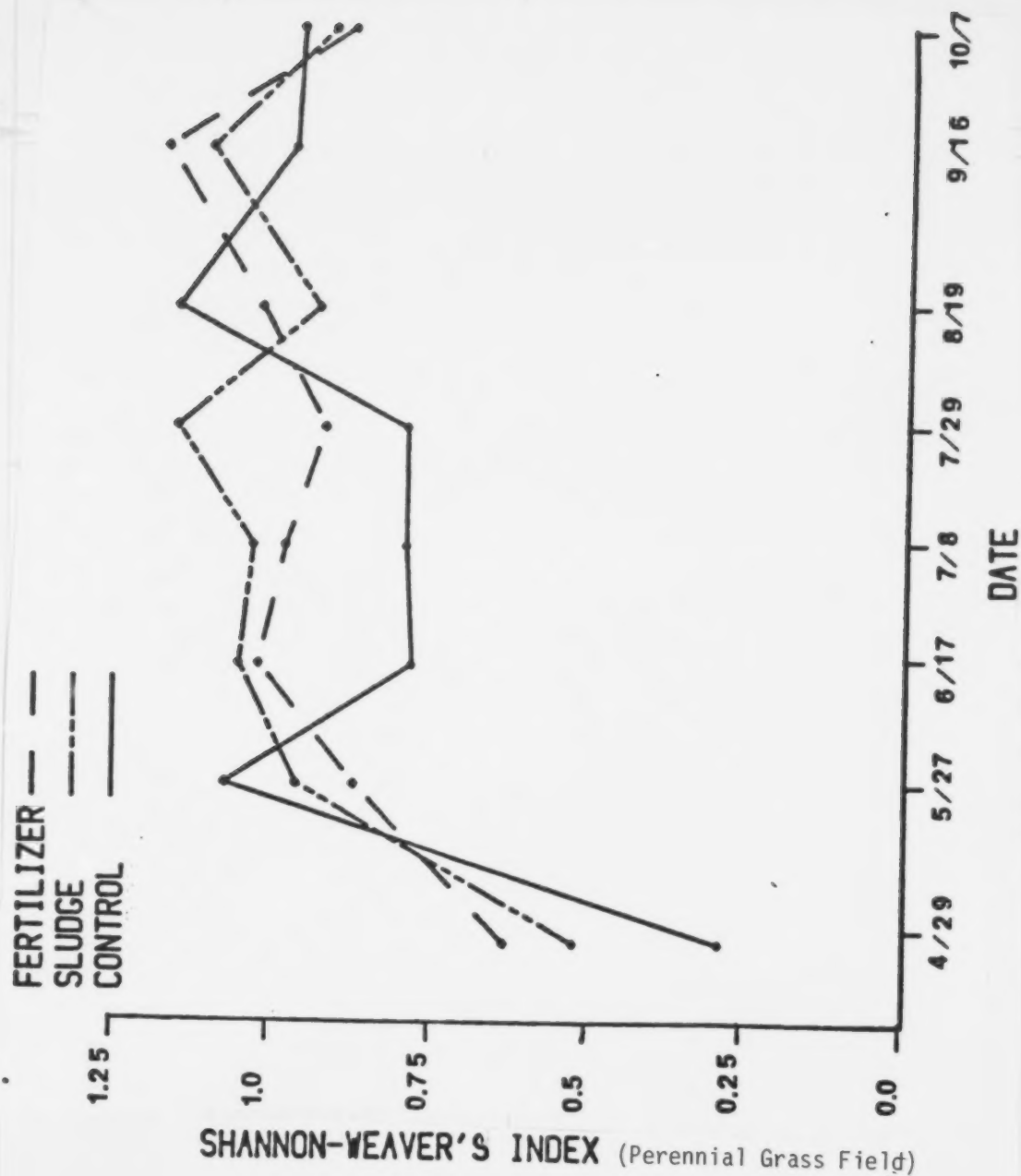


Figure 24. Shannon-Weaver's Index in the perennial grass field.

TABLE 78. PEAK BIOMASS, LITTER, STANDING DEAD AND DAILY PRODUCTIVITY IN THE ANNUAL AND PERENNIAL FIELD. DATE OF PEAK IS SHOWN IN PARENTHESIS. VALUES ARE EXPRESSED AS $g\ m^{-2}$

	Live biomass	Litter	Standing dead	Productivity
<u>Annual Field</u>				
Fertilizer	938 (8 18)	254 (9 15)	507 (10 6)	21 (6 16)
Sludge	613 (8 18)	131 (10 6)	167 (9 15)	16 (8 18)
Control	396 (7 7)	117 (10 6)	178 (10 6)	8 (6 16)
<u>Perennial Field</u>				
Fertilizer	533 (7 29)	211 (6 17)	316 (4 29)	16 (8 19)
Sludge	427 (7 19)	232 (7 8)	333 (4 29)	14 (8 19)
Control	230 (8 19)	309 (7 29)	407 (4 29)	7 (8 19)

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTION
HERE

TABLE 79. PEAK BIOMASS OF SPECIES WITH A PEAK GREATER THAN 5 g m^{-2}
IN THE ANNUAL FIELD. VALUES ARE EXPRESSED AS $\text{g m}^{-2} \text{ YR}^{-1}$. VALUES
IN THE FERTILIZER AND SLUDGE TREATMENTS ARE MEANS OF THREE
REPLICATE PLOTS AND THE CONTROL IS MEAN OF TWO PLOTS

	Fertilizer	Sludge	Control
<u>Ambrosia artemisiifolia</u>	877 [±] 88	456 [±] 42	151 [±] 20
<u>Triticum aestivum</u>	536 [±] 64	326 [±] 25	364 [±] 1
<u>Chenopodium album</u>	56 [±] 26	5 [±] 7	6 [±] 5
<u>Cirsium arvense</u>	48 [±] 43	52 [±] 28	89 [±] 69
<u>Setaria faberii</u>	21 [±] 26	153 [±] 83	8 [±] 3
<u>Trifolium pratense</u>	14 [±] 9	38 [±] 15	110 [±] 40
<u>Polygonum pennsylvanicum</u>	14 [±] 7	10 [±] 7	0
<u>Phleum pratense</u>	10 [±] 13	0	0
<u>Dactylis glomerata</u>	8 [±] 10	8 [±] 11	1-1
<u>Melilotus officinalis</u>	5 [±] 2	18 [±] 8	26 [±] 9
<u>Trifolium hybridum</u>	1 [±] 1	15 [±] 6	5 [±] 2
30 others	100	159	12
Total	1690-146	1240-101	772-5

BEGIN
LAST LINE
OF TEXT

BOTTOM
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLE
AND ILLU-
STRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTIONS
HERE

TABLE 80. PEAK BIOMASS OF SPECIES WITH PEAK GREATER THAN 5 g m^{-2} IN THE PERENNIAL FIELD. VALUES ARE EXPRESSED AS $\text{g m}^{-2} \text{ YR}^{-1}$. VALUES IN THE FERTILIZER AND SLUDGE TREATMENTS ARE MEANS OF THREE REPLICATE PLOTS AND THE CONTROL IS A MEAN OF TWO REPLICATE PLOTS

Species	Fertilizer	Sludge	Control
<u>Pestuca elatior</u>	350 [±] 54	177 [±] 16	125 [±] 18
<u>Phalaris arundinaceae</u>	270 [±] 85	200 [±] 47	119 [±] 4
<u>Poa pratense</u>	120 [±] 14	100 [±] 27	75 [±] 5
<u>Solidago spp.</u>	120 [±] 91	112 [±] 22	21 [±] 13
<u>Dactylis glomerata</u>	105 [±] 86	48 [±] 32	29 [±] 21
<u>Melilotus officinalis</u>	55 [±] 78	2 [±] 2	9 [±] 5
<u>Phleum pratense</u>	15 [±] 12	6 [±] 6	-
<u>Setaria faberii</u>	11 [±] 14	13 [±] 17	-
<u>Alopecurus pratensis</u>	2 [±] 1	13 [±] 3	2-1
17 others	62	14	44
Total	1110-67	684-119	424-52

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTIONS
HERE

TABLE 81. MEAN $\pm$ S.E. FOR GRAIN PRODUCTION IN THE WHEAT FIELD

	Fertilizer	Sludge	Control
Seeds m^{-2}	20717 $\pm$ 1805	14980 $\pm$ 1491	1140 $\pm$ 1077
Heads m^{-2}	1238 $\pm$ 110	864 $\pm$ 179	780 $\pm$ 110
Weight (g m^{-2})	600 $\pm$ 53	426 $\pm$ 48	288 $\pm$ 28
Seeds head $^{-1}$	18 $\pm$ 11	18 $\pm$ 11	16 $\pm$ 13
Seed weight	0.04 $\pm$ 0.09	0.03 $\pm$ 0.002	0.03 $\pm$ 0.001

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTIONS
HERE

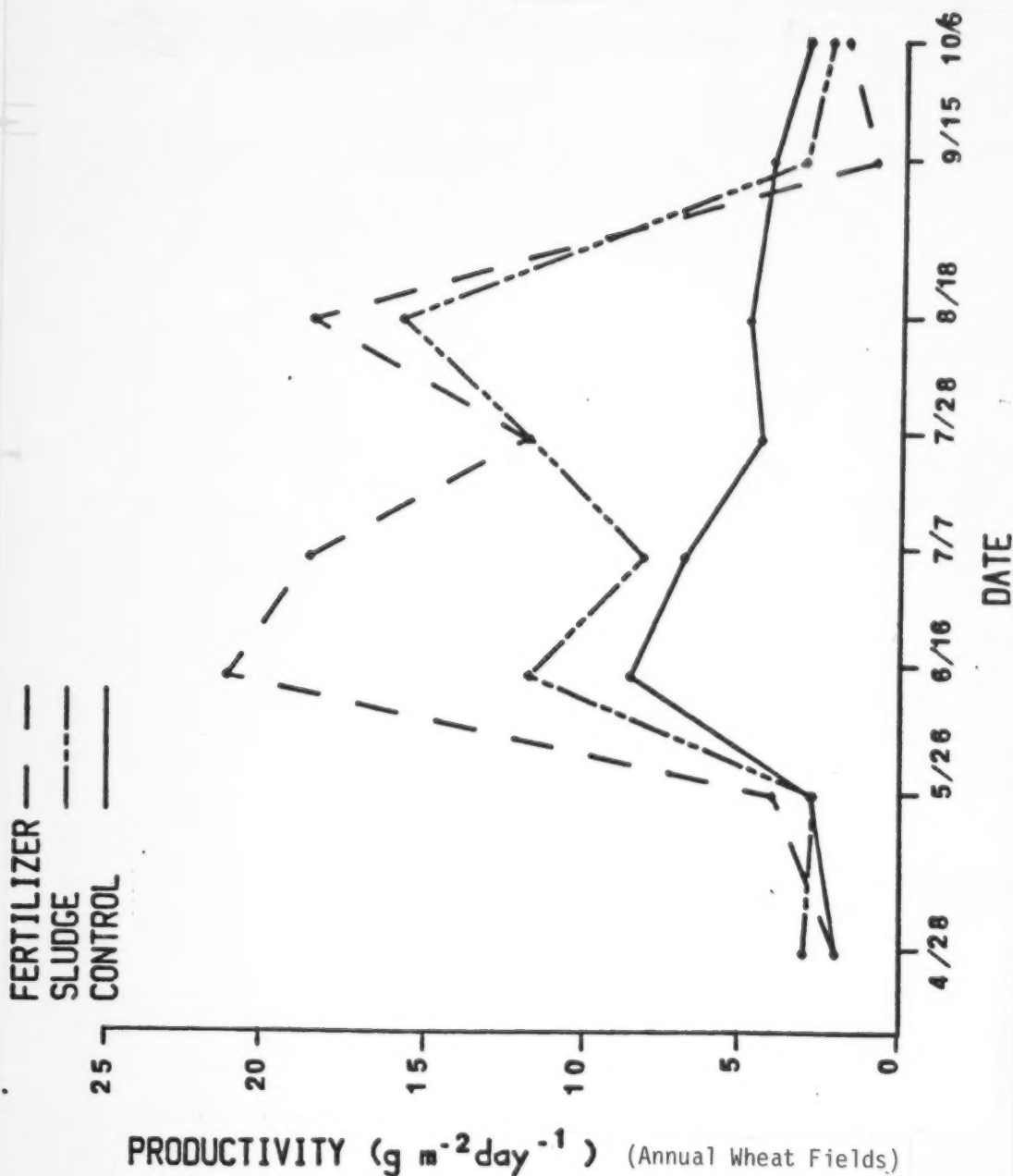


Figure 25. Productivity in the annual wheat field.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

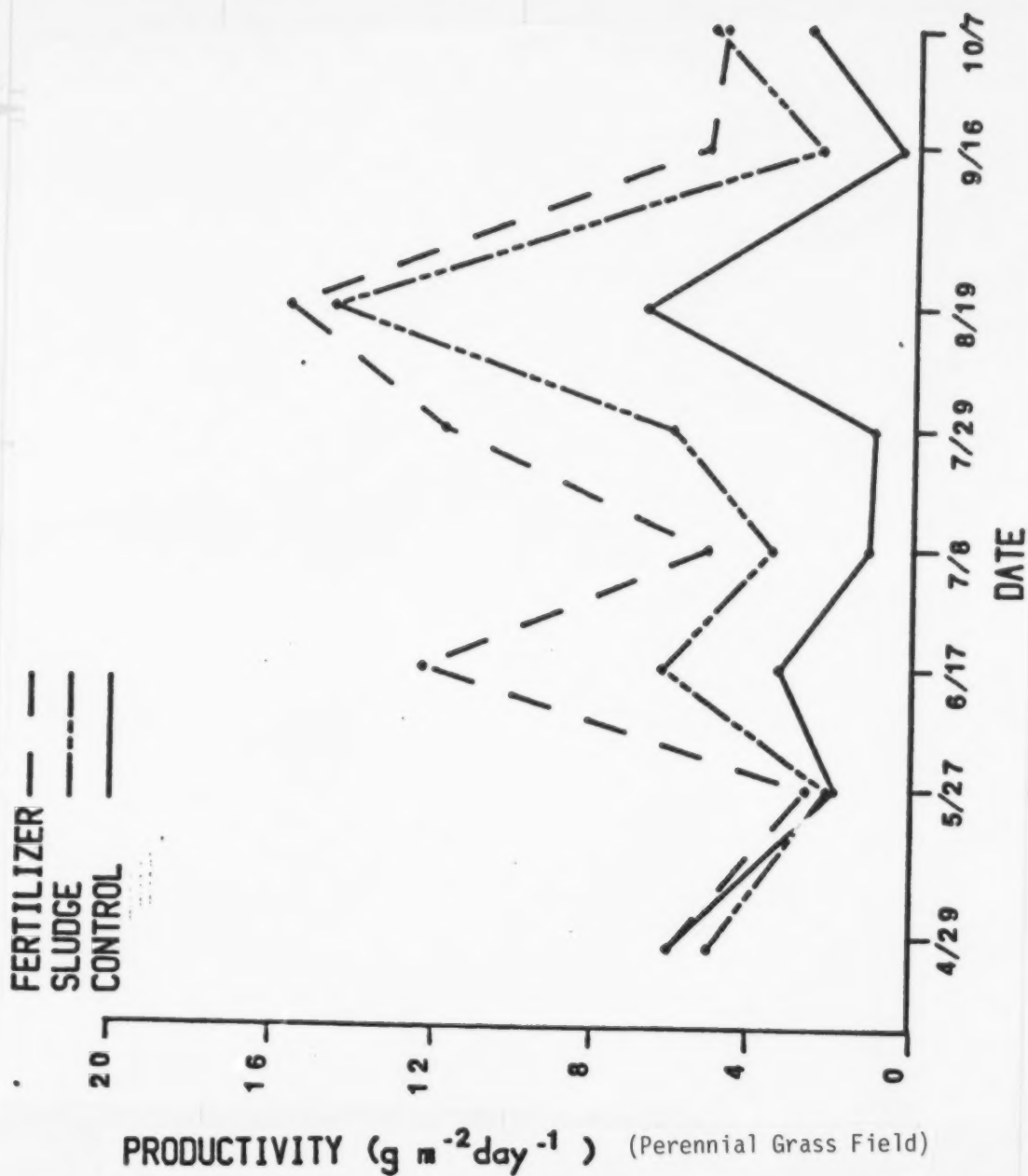


Figure 26. Productivity in the perennial grass field.

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

community types. The opposite was true with respect to apportionment values.

Pathology Report on Voles

A total of 16 experimental groups were brought to the laboratory for examination. Table 82 gives the summary of treatment.

TABLE 82. EXPERIMENTAL GROUPS OF VOLES AND THEIR EXPOSURE

Treatment	Groups
Fertilized agricultural field	01, 02, 04
Sludge agricultural field	03, 07, 08
Control agricultural field	05, 06
Fertilized fallow field	09, 13, 14
Sludge fallow field	11, 12, 15
Control fallow field	10, 16

No pathologic alterations were seen in the lungs and kidneys of the voles -- controls as well as treated (135). The ovaries were also of usual structure. However, some of the animals in each group showed ovaries that had not fully matured, whereas, others showed recent or recent and old corpora lutea. A similar situation was present in the males. Some testes were matured to different degrees. Since the maturation of the gonads reflects the age of the animals, the length of exposure was hard to assess. This was supported to some extent by the histologic findings in the livers, the organ that had cytologic alterations attributable to exposure.

The least severe hepatic change is depicted in Figure 27. The cells were swollen and contained multiple vacuoles. These were filled with a fluid. Their size varied to some extent. The nuclei showed no alterations. This was the most common lesion. It is a hydropic degeneration which is reversible.

A combination of hydropic vacuoles and larger sharply delineated ones is demonstrated in Figure 28. The latter vacuoles appear to be empty; they actually contain lipid material. Again, the hepatic cells are swollen. The boundaries between the cells are obscured. This type of degeneration is more severe and not easily reversible.

The most severe alterations are presented in Figure 29. The hepatic cells are ill-defined. Hydropic and fatty changes are seen. Additionally, some nuclei are small and pyknotic, whereas, others are broken up in pieces. The nuclear membrane is irregular in outline.

The hepatic changes are summarized in Table 83 for those groups of voles that had been living on agricultural fields. The groups were combined

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTIONS
HERE

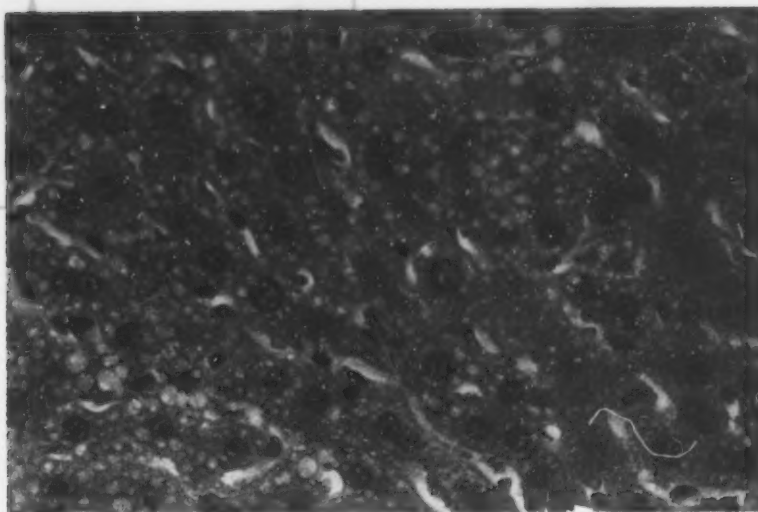


Figure 27.2 Hydropic degeneration of hepatic cells.
Explanation in text. Hematoxylin and
eosin; 250x.

9-1/8"

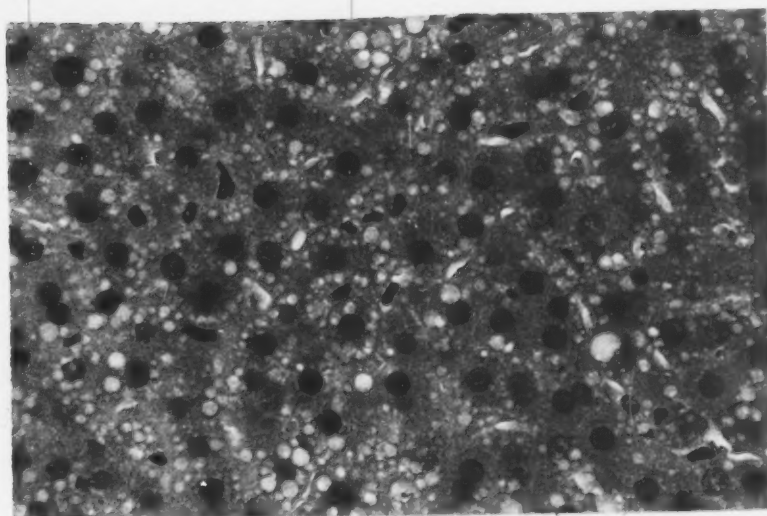


Figure 28. Hydropic and fatty changes in hepatic
cells. Hematoxylin and eosin; 250x.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA;
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

3/8"

182

PAGE NUMBER

TYPING GUIDE SHEET

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD,
BEGIN
SECTIONS
HERE

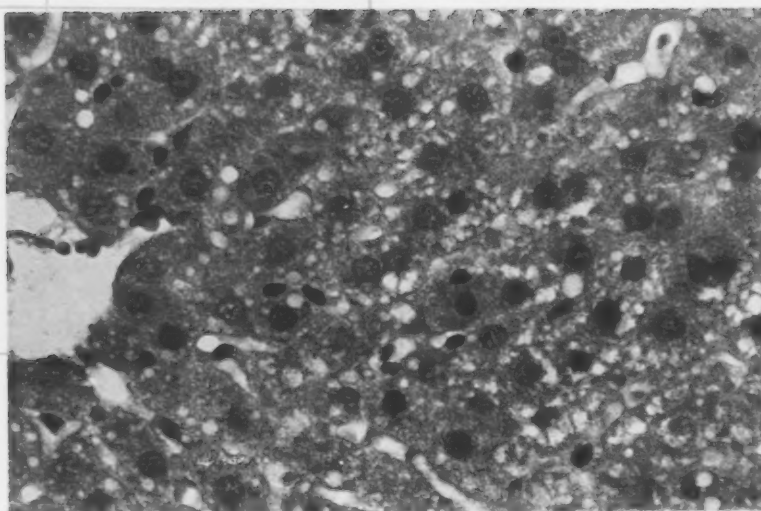


Figure 29. More severe hydropic changes in hepatic cells. Nuclear alterations are present. More detail in text. Hematoxylin and eosin; 250x.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA,
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

since no substantial differences could be detected. The total number of males and females are given. There seems to be no significant difference in the incidence of lesions between fertilized and sludge fields. Generally

TABLE 83. LIVER DEGENERATION IN VOLES ON AGRICULTURAL FIELDS

Treatment	Number of Voles		Hydropic		Fatty		Nuclear	
	Males	Females	♂	♀	♂	♀	♂	♀
Fertilized	22	15	11	8	1	-	6	2
Sludge	18	18	10	9	-	3	2	2
Control	13	11	4	3	1	-	-	-

the alterations are of mild degree. A definite effect is present in the exposed groups over the control. The more severe changes are not present in the untreated voles, especially when one considers that the fatty degeneration occurred in a male without testes.

Table 84 contains the data on voles maintained on fallow fields. Most frequent was the hydropic degeneration. The incidence seemed to be slightly higher in the female sludge groups. The control groups had a significantly lower occurrence of degeneration.

TABLE 84. LIVER DEGENERATION IN VOLES ON FALLOW FIELDS

Treatment	Number of Voles		Hydropic		Fatty		Nuclear	
	Males	Females	♂	♀	♂	♀	♂	♀
Fertilized	19	17	14	7	-	-	3	1
Sludge	18	18	13	11	-	-	-	-
Control	15	6	4	2	-	-	2	-

Comparing the agricultural groups with the fallow ones, there was a lower incidence of severe (fatty and nuclear) cell degenerations in the fertilized fallow field animals (4 degenerations in 36 voles) than in the fertilized agricultural field ones (9 degenerations in 37 voles). Voles on the sludge treated agricultural fields (7 degenerations in 36 voles) show a more severe degeneration than on the sludge-treated fallow fields (no degenerations in 36 voles). One has to conclude that the agricultural fields are slightly more hazardous than the fallow fields to the voles. This, however, should be considered with caution because the endpoint of toxicity was measured only on the pathology in the livers. A more sound evaluation could have been done on the basis of pathohistologic examination of all organs.

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

Meadow Vole Populations

DROPPED
HEAD
BEGIN
SECTIONS
HERE

Meadow vole (*Microtus pennsylvanicus*) populations were monitored two years by live-trapping (136). Vole population densities were affected by treatment only in the first year wheat fields, where sludge treatments had higher densities than any of the other treatments in the two years (Figures 30 and 31). The fertilizer treatments in the first year wheat fields had low densities due to poor habitat quality as a result of domination of the plant community by ragweed. In the wheat field vole populations responded to changes in plant community structure induced by fertilization, but showed no apparent negative reaction to the sludge per se. The data points shown are the means of the treatment replicates on each trapping date. The sludge plots were significantly higher than the fertilized plots and controls after 21 August (Duncan's multiple range test at the 95% level of significance). After 4 September all three treatments were significantly different from each other. Although the fertilized plots had the highest Net Primary Productivity (NPP) they were so dominated by ragweed after the wheat had matured that much of the ground cover was shaded out, resulting in poor vole habitat. Ragweed made up 52% of NPP in the fertilized plots, and no other species besides wheat comprised greater than 3% of NPP. These plots had the lowest Shannon-Weaver diversity of the three treatments. In the sludge plots ragweed comprised only 34% of NPP, and foxtail and red and sweet clover were relatively productive as ground cover. Ragweed comprised 15% of NPP in the control plots. Vole populations were highest in the sludge plots because the fertilizing effect of the sludge increased plant production, but at a slower rate than the fertilizer, allowing lower-growing species to become established and compete with the ragweed. Survivorship, longevity, percentage of breeding adults, and recruitment rates were not affected by sludge treatment. Adult sex ratios favored females in nutrient-subsidized treatments and males in controls. This appears to have been related to differences in food quality. Juvenile sex ratios were equal.

There were no treatment differences in tissue concentration of Pb, Zn, or Cu (135). Cd concentrations were elevated in kidneys and livers of all sludge-treated voles. The mean liver and kidney cadmium concentrations in voles from the control, fertilized and sludge-treated fields for 1978 and 1979 are shown in Tables 85 and 86. The differences between males and females, and between age groups are also included in Tables 85 and 86. Cd concentrations were high in the kidneys of all sludge-treated voles regardless of community-type. Although vole population dynamics were not adversely affected, there exists the possibility for long-term, low-dose Cd effects in sludge-treated plots. No organ or whole weight differences were noted between treatments. There was slight liver pathology in voles from both nutrient-subsidized treatments. This may have been due to social stress and not directly related to treatment. No treatment differences were observed in learning ability or memory as measured by the swim-escape test. The mid-successional old-fields appear to be preferable to the early stages of succession or to the agricultural fields as land application sites for sludge disposal in terms of minimizing effects on small mammal populations.

BEGIN
LAST LINE
OF TEXT

In the old-field the plant species showed no differences in diversity or composition between treatments as was previously discussed. Vole populations

3/8"

185

BOTTOM OF
IMAGE AREA.
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

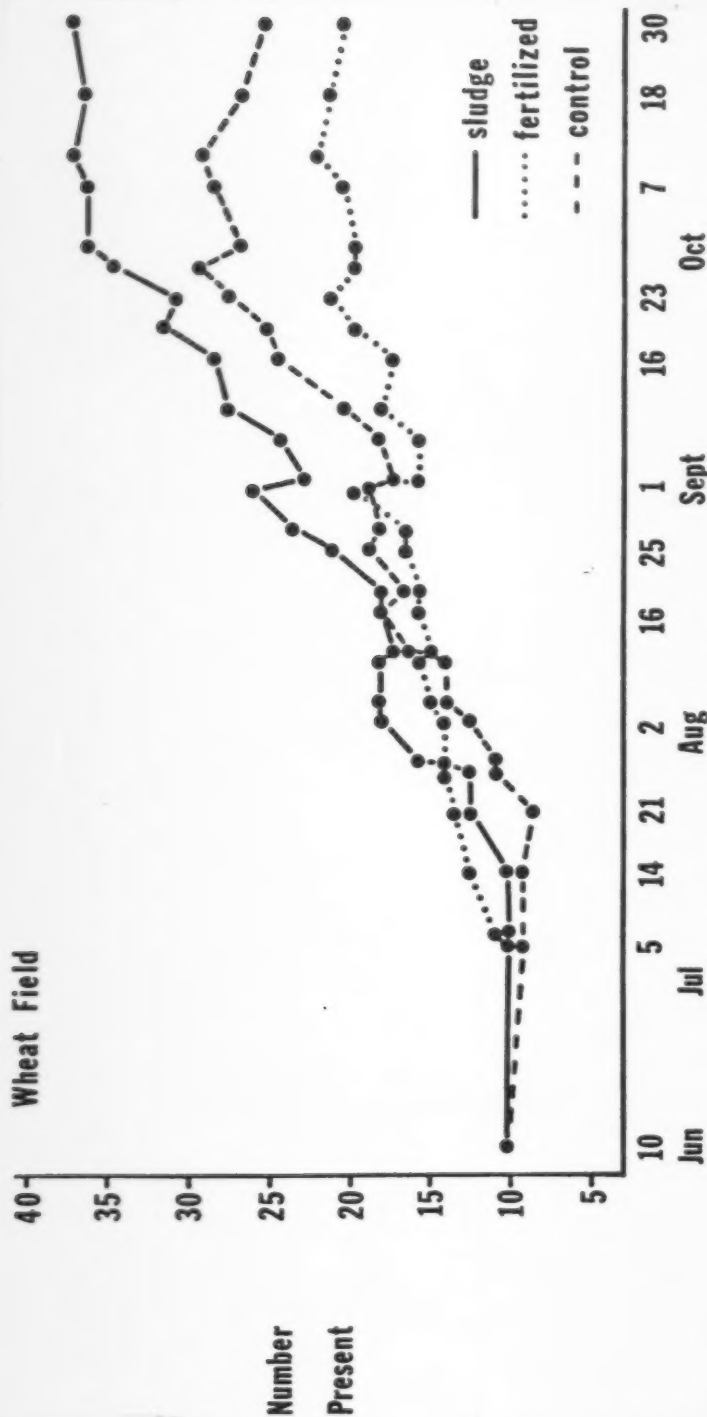


Figure 30. Meadow vole population densities in the wheat field.

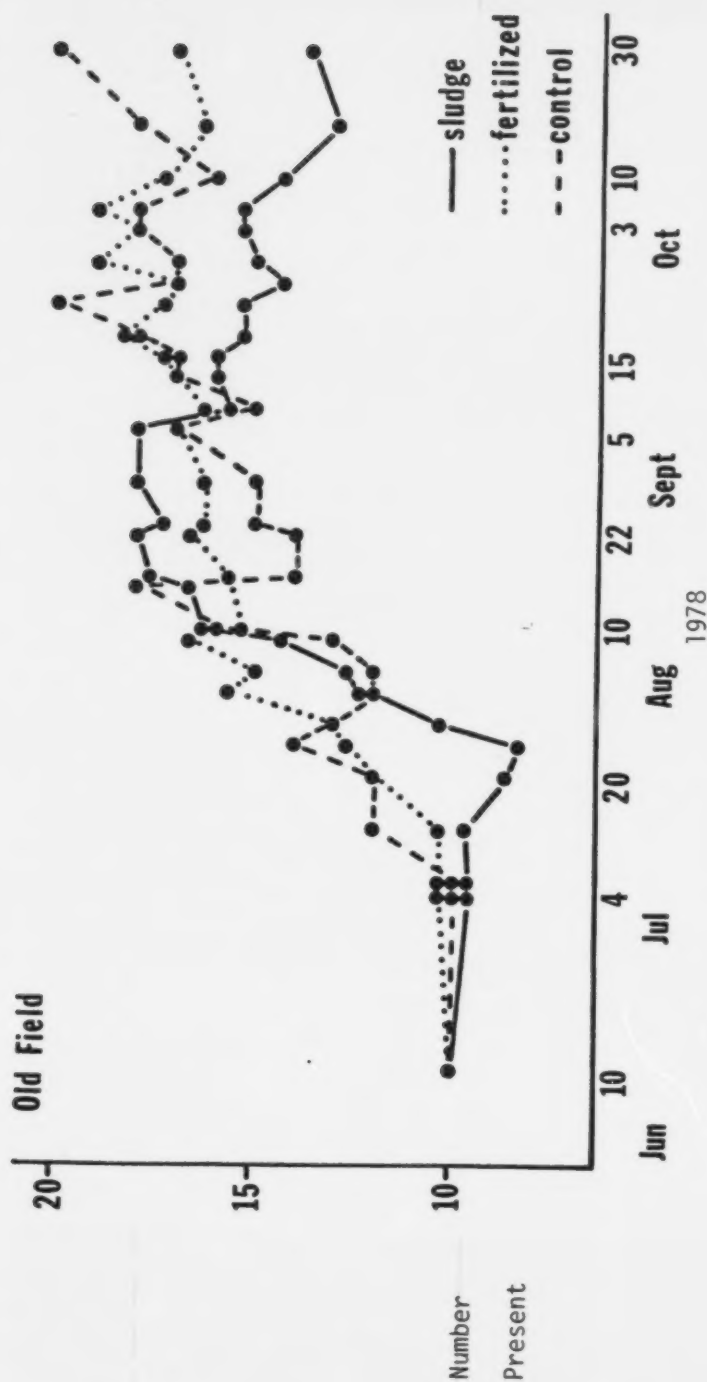


Figure 31. Meadow vole population densities in the old-field.

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD,
BEGIN
SECTIONS
HERE

TABLE 85. CADMIUM CONCENTRATIONS* IN VOLES FROM THE WHEAT FIELD (1978) AND FIRST-YEAR OLD-FIELD (1979) ($\mu\text{g/g}$ WET WEIGHT)

	Control		Fertilized		Sludge	
	1978	1979	1978	1979	1978	1979
Liver						
adult males	0.01 (3) e	0.03 (3) e	0.03 (5) e	0.04 (3) e	0.14 (4) cde	0.28 (4) cd
adult females	0.04 (4) e	0.06 (2) de	0.09 (5) de	0.09 (4) de	0.56 (5) b	0.96 (4) a
sub-adult males	0.01 (4) e	0.02 (3) e	0.03 (6) e	0.05 (5) e	0.20 (5) cde	0.47 (4) b
sub-adult females	0.02 (3) e	0.04 (2) e	0.04 (4) e	0.04 (3) e	0.29 (6) e	0.83 (4) a
Kidneys						
adult males	0.01 (1) f	0.16 (2) f	0.09 (1) f	0.31 (2) f	0.90 (3) def	1.72 (3) bed
adult females	0.20 (2) f	0.59 (2) ef	0.31 (1) f	0.88 (3) def	2.16 (3) bc	3.59 (4) a
sub-adult males	0.01 (2) f	0.12 (2) f	0.06 (1) f	0.10 (1) f	0.63 (2) ef	1.71 (3) bed
sub-adult females	0.05 (2) f	0.14 (1) f	0.04 (2) f	0.17 (1) f	1.37 (3) cde	2.34 (3) b

*Means of treatment replicates; the number of samples is in parentheses; means within each organ followed by the same letter are not significantly different, between different age-sex groups and years, and among the three treatment fields ($p > 0.05$, Duncan's multiple range test).

BEGIN
LAST LINE
OF TEXT

3/8"

188

PAGE NUMBER

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

TABLE 86. CADMIUM CONCENTRATIONS* IN VOLES FROM THE FOURTH-YEAR (1978) AND FIFTH-YEAR (1979) OLD-FIELDS ($\mu\text{g/g}$ WET WEIGHT)

	Control		Fertilized		Sludge	
	1978	1979	1978	1979	1978	1979
Liver						
adult males	0.02 (4) d	0.04 (2) cd	0.03 (7) d	0.04 (4) cd	0.17 (5) cd	0.27 (4) bc
adult females	0.26 (1) bc	0.44 (3) b	0.11 (6) cd	0.12 (4) cd	0.95 (5) b	1.06 (4) a
sub-adult males	0.11 (3) cd	0.02 (3) d	0.01 (4) d	0.06 (5) cd	0.07 (4) cd	0.44 (4) b
sub-adult females	0.14 (4) cd	0.04 (3) cd	0.05 (4) cd	0.07 (4) cd	0.23 (4) bc	0.40 (4) b
Kidneys						
adult males	0.15 (1) e	0.40 (2) de	0.14 (3) e	0.44 (3) de	0.81 (3) cde	1.40 (3) bc
adult females	-	1.61 (2) bcde	0.59 (1) cde	1.30 (3) bcde	2.27 (3) b	6.57 (3) a
sub-adult males	0.15 (2) e	0.01 (2) e	0.34 (2) de	0.33 (3) e	0.39 (2) de	1.93 (1) bc
sub-adult females	0.70 (2) cde	0.16 (2) e	0.12 (2) e	0.55 (2) cde	1.10 (2) bcde	2.24 (3) b

*Means of treatment replicates; the number of samples is in parentheses; means within each organ followed by the same letter are not significantly different, between different age-sex groups and years, and among the three treatment fields ($p > 0.05$, Duncan's multiple range test).

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD:
BEGIN
SECTIONS
HERE

were not significantly different until after 18 September, when the sludge plots were slightly lower than the fertilized and controls. Since there were no distinct differences in the plant communities between treatments this may reflect a negative sludge effect. However it is also very possible that this is due to slight differences in plant species composition, or to chance.

In summary, vole populations in the wheat fields showed no apparent negative response to sewage sludge application. Populations in the sludge plots were higher than controls due to the fertilizing effect of the sludge. Populations in the fertilized plots were low due to sparse ground cover resulting from ragweed dominance. Vole populations in the old-fields responded similarly in all treatments, except for a slight decline in the sludge populations in late summer. In comparing between communities these results reflect the greater stability of the old-fields and the ability of the perennial grasses to resist domination by opportunistic annuals. Research is continuing along several lines in an attempt to more completely characterize the effect of sewage sludge application on mammal populations.

6-1/2"

9-1/8"

BEGIN
LAST LINE
OF TEXT

3/8"

190

BOTTOM OF
IMAGE AREA.
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD;
BEGIN
SECTIONS
HERE

SECTION 12

POTENTIAL HEALTH EFFECTS FROM VIABLE AND NONVIABLE EMISSIONS
ASSOCIATED WITH SLUDGE INCINERATION AND PYROLYSIS

INTRODUCTION

Combustion is a rapid exothermic oxidation of combustible substances. It is a simple and a quick way of reducing the bulk of human wastes. The principal methods for thermal oxidation or degradation of wastewater sludge now in use or under serious development are incineration (conventional multiple-hearth furnace or fluidized bed), wet oxidation and pyrolysis. In some circumstances, sludge may be combined with municipal refuse, for co-incineration or co-pyrolysis. The principal characteristics of these methods are summarized in Table 87 (137,138). Combustion of sludge is a two-step process involving drying followed by burning. It is usually accomplished by feeding waste into an incandescent chamber (furnace). However, it is usually difficult to burn sludge because of its higher moisture content and the tendency of the wet sludge to resist mixing with combustion air. The primary products of combustion are water, CO₂ and ash. The advantages of combustion or thermal degradation of sludge include:

- a) nearly complete combustion of organics
- b) large reduction of sludge volumes
- c) relatively easy disposal of an inert ash, and
- d) the destruction of pathogenic microorganisms.

However, if not properly controlled, thermal degradation can cause air pollution because of smoke, odor and dust emissions.

Incineration is a thermal degradation of a substance in the presence of oxygen at high temperature and has been used by many cities for a number of years. Drying and combustion may be accomplished in separate units or successively in the same unit, depending upon temperature constraints and control parameters. Incineration is complete combustion. It is a complex process involving thermal and chemical reactions which occur at varying times, temperatures, and locations in the furnace. Multiple hearth furnaces are the most widely used sludge incinerators in the United States.

Pyrolysis is a destructive distillation of sludge in the absence of oxygen at temperatures somewhat lower than those needed for incineration. In the pyrolysis process, the volatile matter will distill leaving carbon and inert residue behind.

BOTTOM OF
IMAGE AREA,
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

BEGIN
LAST LINE
OF TEXT

3/8"

191

PAGE NUMBER

TABLE 87. CHARACTERISTICS AND POTENTIAL IMPACTS OF THERMAL REDUCTION METHODS (137,138)

Process	Theory and operation	Comments	Potential impacts
Multiple hearth incineration	Counter-current downward flow by gravity through hot combustion gases from oil or gas burners. Rabble arms gently agitate thin sludge layers on each successive hearth to promote drying, then burning.	Consumes scarce oil or gas. Inherently entrains fine flyash in upward-flowing flue gases. Counter-current principle very effective for drying wet sludge prior to ignition	Fine flyash emission which should be controlled by available dust collection.
Flash drying incineration	Spraying of sludge into large drying chamber enables subsequent choice of either burning or utilization of dried sludge as low-grade fertilizer.	Justified only where markets for low-grade fertilizer seem promising.	Same as above.
Fluidized bed incineration	Upward flow of combustion air through incandescent bed of inert particles gives excellent mixing, good particle-gas contact.	Inherently entrains fine flyash in upward-flowing flue gases.	Same as above.
Wet oxidation	Use of elevated pressure and temperature in closed reactor tank to promote oxidation of sludge.	Reduces volatilization of metals and organics in sludge. Operation requires uncommon skill.	Minor.

(continued)

BEGIN
FIRST
LINE OF
TEXT
HERE

DROPPED
HEAD
SECTION
HERE

CENTER OF
PAGE

TOP OF
IMAGE
AREA

TABLE 87 (continued)

Process	Theory and operation	Comments	Potential impacts
Combined refuse/sludge	Mixing of sludge with municipal refuse and then burning mixture on travelling or moving grate.	Many full scale attempts have failed primarily from problems of nonuniform burning because of difficulty of mixing the two dissimilar components.	Same as incineration above.
Pyrolysis	Thermal decomposition of carbonaceous and organic material in a deficiency of air.	Until demonstrated successfully on a full scale plant, the viability of this process for sewage sludge remains uncertain.	Reduce flyash emission less volatilization of metals; smoke and organic emissions have caused some problems.

Reprinted from "Multimedidum Management of Municipal Sludge," by National Academy of Sciences, National Research Council, Analytical Studies for the U.S. Environmental Protection Agency, Volume IX, 1978, with permission of National Academy of Sciences (138).

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

The potential health effects to sludge disposal workers and the general population from exposure to pathogens, gaseous and particulate emissions and ash from incineration and pyrolysis, are considered in this section.

INCINERATION

Incineration converts the organic fraction of the sludge into primarily CO_2 and H_2O , and the inorganic fraction into vapors and ash. The ashes (mostly containing heavy metal oxides) are usually disposed by landfilling. Approximately 35% of total sewage sludge is currently incinerated at over 100 facilities in the United States. There are several types of sludge incinerators commercially available. They are multiple hearth furnace, fluidized-bed furnace, electric furnace and single hearth cyclone furnace. In all furnaces, the drying and combustion processes follow the same phases - raising the temperature of the feed sludge to 212°F (100°C), evaporating water from the sludge, increasing the temperature of the water vapor and air, and increasing the temperature of the dried sludge volatiles to the ignition point. They differ primarily in the internal arrangement of the incinerator for the reception and exposure of the material to be burned. The multiple-hearth furnace is the most popular type of sludge incinerator in the United States. In this system, sludge is passed through three zones or hearths. Incineration temperatures range from 800° to 1000°F in the top hearth where the sludge is dried; $1,400$ to $1,800^\circ\text{F}$ in the middle hearth where volatile gases and solids are burned; and 500 to 600°F in the bottom hearth. A small proportion of sludge incinerators are fluidized-bed incinerators in which sludge is introduced into a bed of sand which has been preheated to approximately $1,300$ to $1,500^\circ\text{F}$. Combustion in a multiple-hearth incinerator can destroy nearly 100% of the organics. Disadvantages generally involve capital cost, ash, odor nuisance and possible explosions.

Sludge parameters that are most important to incineration are moisture, volatile materials, inert materials and thermal value (139-141). Of these, moisture is particularly important because of the thermal load it imposes on the incineration process. Moisture content of sludge is usually reduced by mechanical dewatering techniques prior to incineration. Volatile and inert materials affect the heat value of the sludge. Almost all of the combustible substances in sludge are volatile.

Incineration has the advantage of destroying the organic matter present in sludge, leaving only an odorless sterile ash, as well as reducing the solid mass by about 90%. Disadvantages include the ash disposal problem and the potential for air pollution. During the wastewater treatment, the heavy metals are concentrated in the sludge unless removed prior to incineration. The metals are not destroyed by incineration but are redispersed into the atmosphere and also concentrated in the ash. When the ash is landfilled, these metals may get into groundwater through leaching.

Trace metal composition of sewage sludges from 10 cities in the United States is shown in Table 88 (142). As can be seen, the concentration of heavy metals in sewage sludge varies widely from treatment plant to treatment plant. The concentration also varies with time.

BEGIN
LAST LINE
OF TEXT

3/8"

194

BOTTOM OF
IMAGE AREA;
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

TABLE 88. ANALYSIS OF TRACE ELEMENTS IN MUNICIPAL SLUDGES FROM 10 U.S. CITIES (6)^a
(ppm, dry weight basis)

Element	City									
	Atlanta	Chicago	Houston	Los Angeles	Miami	Milwaukee	New York	Philadelphia	San Francisco	Seattle
Arsenic	3.6	29	20.4	26	10	3.3	8.0	16.2	6.4	30
Barium	535	623	485	1066	938	344	417	533	542	504
Cadmium	104.2	14.8	111.8	171.4	149.6	443.7	29.7	192.4	8.5	64
Chromium	1320	207	3480	4925	1430	14,000	646	2320	1500	1320
Cobalt	9.4	3.7	6.3	16.8	17.6	4.3	6.2	15.6	5.3	9.4
Copper	1463	578	1560	2890	1200	1288	1890	2680	900	1170
Mercury	6.9	6.1	3.8	7.1	15.5	3.4	15	4.7	18	8.2
Manganese	267	95	102	116	32	134	152	95	113	350
Molybdenum	6.0	10.8	9.8	40	37	32.9	5.0	8.1	2.2	2.3
Nickel	169	51.2	102	402	453	360	140	432	223	153
Lead	1445	605	2236	3065	1467	2253	1976	7627	2521	2411
Zinc	2838	1160	2560	4590	1400	1370	1340	6890	601	1830

^aAdapted from A. K. Furr et al. Multielement and Chlorinated Hydrocarbon Analysis of Municipal Sewage Sludges of American Cities. Env. Sci. and Tech. 10(7):683-687, 1976 (142).

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD;
BEGIN
SECTIONS
HERE

Incineration poses two potential sources of risks to public health - 1) air pollution from increased ambient air concentration of gaseous and particulate matter due to emissions from sludge combustion, and 2) possible groundwater contamination from heavy metal leachates from landfilled ash. The rate of emissions from an incinerator varies widely depending on the quantity and composition of the sludge, type of the incinerator and the efficiency of the air pollution control equipment. The degree of public health hazard posed by emissions from an incinerator will be site-specific and will depend on the nature of emissions, local meteorological conditions, ambient air quality levels, etc.

Air pollution is a major concern in any combustion process and may be the most difficult and expensive environmental consideration to correct. Incineration might contribute to air pollution because of incomplete combustion and formation of intermediate combustion products (143). The U.S. Environmental Protection Agency (EPA) has established standards of performance for municipal solid waste incinerators and wastewater sludge incinerators (139). These guidelines indicate that new sludge furnaces will have to comply with the air pollution standards.

Air Pollution Standards

National Ambient Air Quality Standards (NAAQS)-State Implementation Plans (SIP)--

Federal air quality regulations are derived from the Clean Air Act Amendments of 1970, the Energy Supply and Environmental Coordination Act of 1974, and most recently, the Clean Air Act Amendments of 1977. The NAAQS established threshold levels of air pollutants below which no adverse effects would occur. These levels were designed to provide an adequate margin of safety so as to protect the public health.

Air pollutants are classified into two groups: primary pollutants and secondary pollutants. Primary pollutants are those emitted directly from sources, while secondary pollutants are formed by chemical and photochemical reactions of primary pollutants with the atmosphere. Primary pollutants include carbon monoxide (CO), hydrocarbons, oxides of nitrogen (NO_x), sulfur dioxide (SO₂), total suspended particulates (TSP) and lead (Pb). Photochemical oxidants and nitrogen dioxide (NO₂) are the principal secondary pollutants. The quantity of secondary pollutants is dependent on the availability of sunlight as much as on the availability of primary pollutants.

The 1970 Amendments to the Clean Air Act required each state to develop its own State Implementation Plans (SIP) to meet the federal standards by 1975 or 1977, the date dependent on the severity of the state air quality problems. For areas already meeting NAAQS standards, implementation plans must include a program to prevent significant deterioration of air quality.

National Emission Standards for Hazardous Air Pollutants (NESHAPS)--

Subpart A of NESHAPS (40 Code of Federal Regulations (CFR)) (63) comprises general provisions covering definitions, applications, reporting, and

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

3/8"

196

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD.
BEGIN
SECTIONS
HERE

waivers. Subpart E deals with mercury emissions and applies to all operations that burn or dry wastewater sludge. The NESHAPS standard is currently seven pounds of mercury (3.2 kg) per 24-hour period for any source (144).

Standards of Performance for New Stationary Sources (NSPS)--

Subpart A of NSPS (40 CFR 60) involves general provisions covering definitions, performance tests, authority, and monitoring requirements. Subpart O is applicable to incinerators that burn municipal wastewater sludge and requires that particulates discharged cannot be in excess of 1.30 pounds per ton (0.65 kg/t) of dry sludge feed and that the gas discharged shall not have more than 20 percent opacity (145).

New Source Review Standards (NSR)--

This regulation, 40 CFR 51.18, requires a preconstruction review of all new or modified stationary sources to determine if the source will meet all applicable emission requirements of the State Implementation Plans and the USEPA's Emission Offset Policy.

State and local authorities may mandate a stricter criterion. In addition, the lowest achievable emission rate is required for any regulated source that mandates Best Available Control Technology (BACT).

Prevention of Significant Deterioration (PSD)--

This regulation, 40 CFR 52.21, limits increases in particulate and sulfur dioxide concentrations to specified increments above base levels measured in attainment areas. Data on total emissions for the entire air basin are required in order to evaluate incremental increases in specific emissions due to operation of any new or modified furnaces. If the potential emission rate of a regulated pollutant(s) exceeds 250 tons per year and the allowable emission rate exceeds 50 tons per year then this regulation must be used and public notice is required.

Emissions From Incineration

The mixing of air with sludge at high temperature in the incinerator allows the noncombustible fraction of sludge to become airborne and to be carried away in the exhaust gases. Thus sludge incineration emits significant quantities of fine-grained fly ash (146). In order to be able to meet the air quality standards all sludge incinerators must be equipped with scrubbers. Particulate and gaseous emissions for sludge incinerators with and without scrubbers are listed in Table 89 (138,147). Emission levels for sludge incinerators with scrubbers may not necessarily meet air quality standards because the emissions are a function of the collector efficiency. Several studies have been conducted in recent years on the emission levels and rates from a number of sludge incinerators. Some of the findings are summarized below briefly.

Gaseous Emissions-- Gaseous pollutants that could be released into the atmosphere are SO₂, nitrogen oxides (NO_x), carbon monoxide (CO) and hydrochloric acid (HCl). The sulfur content in municipal content is relatively low, less than 0.1% on a dry weight basis, hence SO₂ emission is not expected to be a serious problem. The temperatures employed in sludge incinerators

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

3/8"

197

PAGE NUMBER

BEGIN
FIRST
LINE OF
TEXT
HERE

CENTER OF
PAGE

TOP OF
IMAGE
HERE

TABLE 89. EMISSION FACTORS FOR SEWAGE SLUDGE INCINERATORS (2,11)

Pollutant	Emissions (per unit of dry solids fired)			
	Uncontrolled		After scrubber	
	lb/ton	kg/MT	lb/ton	kg/MT
Particulates	100	50	3	1.5
Sulfur dioxide	1	0.5	0.8	0.4
Carbon monoxide	Neg	Neg	Neg	Neg
Nitrogen oxides (as NO ₂)	6	3	5	2.5
Hydrocarbons	1.5	0.75	1	0.5
Hydrogen chloride gas	1.5	0.75	0.3	0.15

Reprinted from "Multimedia Management of Municipal Sludge," by National Academy of Sciences, National Research Council, Analytical Studies for the U.S. Environmental Protection Agency, Volume IX, 1978, with permission of National Academy of Sciences (138).

BEGIN
LAST LINE
OF TEXT

REPRINTED BY
EPA/600/4-80/004
CONTAINS
DIMENSION
FOR TABLE
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD;
BEGIN
SECTIONS
HERE

are too low to form appreciable NO_x , no more than 200 ppm. Carbon monoxide is not considered to be a problem if the incinerator is properly operated. The emission of HCl depends on the amount of combustible plastics in the sludge. Average levels of approximately 1.0 ppm - 10.0 ppm have been reported for HCl (146). There are no federal standards for SO_2 and NO_x emissions from sludge incinerators at present.

Toxic Organic Chemicals--Toxic organic chemicals can be released from sludge incineration because of the presence of pesticides and other persistent organic compounds in sludge. A number of these compounds may concentrate in the sludge at wastewater treatment plants. Gabler and Neyland (148) have shown that pesticides and other organic chemicals can satisfactorily be destroyed in a multiple-hearth incinerator.

In a study of incineration of sludge containing low levels of PCBs (1.2 to 2.5 ppm), PCBs were not found in the ash nor in the inlet or outlet scrubber water. The study showed that 99% of PCBs were destroyed at 1600° to 1800°F in 2.0 seconds. Ninety-five percent destruction of PCBs was achieved in a multiple-hearth furnace with no afterburning at normal exhaust temperatures of about 700°F. The study also showed that complete destruction of PCBs was possible when oxidized with an exhaust gas temperature of about 1100°F (146).

Particulates--Available data indicate that on the average stack gases of uncontrolled sludge incinerators contain about 1.0 gr/dscf (grains of particulates per standard cubic foot of dry gas) or about 45 lbs of particulates per ton of sludge burned (141). The federal standard for discharge of particulates from new and modified municipal sludge incinerators requires that particulates discharged cannot be more than 0.65 g/Kg dry sludge input (1.30 lb/ton dry sludge input) (145). This standard applies only to incinerators constructed after March 1974 when the standard became effective. Particulate control devices must be able to remove approximately 96 to 97 percent of particulates in incinerator emissions in order to be able to comply with the standard (148). Many incinerators constructed before the standard became effective discharge considerably higher levels of air pollutants. In many cases, present sludge incinerator particulate control systems do meet local and state particulate standards, which are more stringent than the federal standards.

Heavy Metals--Heavy metals are concentrated in sludge during wastewater treatment. They are not removed by incineration. They are, however, converted into the less soluble oxide form. Metals that are of most concern are zinc, cadmium, lead and mercury. Some metals such as lead, mercury and perhaps cadmium volatilize at sludge incineration temperatures and therefore can be emitted in gaseous and particulate forms. Even with efficient air pollution control devices some volatile metals and particulates will be emitted because of their extremely small submicron sizes. But the impact of these emissions on the ambient environment is considered to be relatively small because their initial quantities in sludge are small (138).

BEGIN
LAST LINE
OF TEXT

Mercury--Mercury presents special problems during incineration. High combustion temperatures decompose mercury compounds to volatile mercuric

BOTTOM OF
IMAGE AREA;
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

3/8"

199

PAGE NUMBER

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD,
BEGIN
SECTIONS
HERE

oxide or metallic mercury. The Federal emission standard for mercury as a hazardous air pollutant resulting from sludge incineration established in 1975 limits mercury emission to a maximum of 3,200 g/24-hr period (0.29 lb/hr) (144). Several studies have been conducted to determine the percentage of mercury in sludge that leaves the incinerator in the stack gases (146, 149, 150), which indicate that anywhere from 68 to 98 percent of mercury in sludge will enter the atmosphere. Using an average emission rate of 1.65 g mercury/metric ton dry solids (146), Los Angeles/Orange County Metropolitan Area (LA/OMA) engineers (151) calculated that a facility processing 1,939 metric tons of sludge per day would approach the limit. No existing or planned incinerators approach this size. Thus it is doubtful that any sewage sludge incinerator would emit more than the federal limit of 3200 gms in 24 hours (152).

Soldano et al. (153) have measured airborne concentrations of inorganic and organic mercury compounds near wastewater treatment facilities in several cities. Both inorganic and organic mercury compounds were detected in a wide range of concentrations. Although concentrations in downwind samples were usually higher than those found in upwind samples, frequently concentrations increased as downwind distance from the plants increased. Concentrations of inorganic mercury were lower than that of organic mercury. In Louisville, KY, and in Memphis, TN, the levels of both inorganic and organic compounds were found to be considerably higher than the U.S. Environmental Protection Agency upper limit of 1000 ng/m³ of air. In addition, upwind samples often had levels as high or higher than downwind to the plant. The data presented by Soldano, therefore, suggest a source or sources in addition to the wastewater treatment plants. The presence of the power plants near the wastewater treatment plants in some cities and other industrial plants located in the area are probably the sources of the higher mercury levels found in the ambient air. The report did not specify whether the plants surveyed had sludge incinerators but it is known from the list of the plants that some of them do not have incinerators.

Dewling et al. (157) have conducted field studies to measure the emission of heavy metals at the fluidized bed sludge incinerator, operated by the Northwest Bergen Sewer Authority, Waldwick, NJ. Their study showed that about 98% of the mercury in the feed sludge was emitted to the atmosphere when combustion temperature, averaged 788°C and only 0.4% was retained in the incinerator ash. Although most of the mercury contained in the feed sludge was emitted into the atmosphere, the Northwest Bergen facility was not found to violate the EPA mercury emission limit.

Lead--From a study of emissions from three sludge incinerators, Farrell et al. (154) showed that under certain conditions as much as 30% of the lead in sludge can pass through air pollution control devices as fine particulate matter. Using a plume dispersion model they calculated that the ground level concentrations of lead from the incinerators were the same order of magnitude as the annual average ambient concentrations in the cities where the plants were located. It should be noted that all three incinerators under study were installed before federal New Source Performance Standards went into effect.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA,
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

Typing Guide Sheet

CENTER
OF PAGE

TOP OF
PAGE

DELETED
HEAD
BEGIN
SECTION
HERE

Whitmore and Durfee (150) showed that less than 15% of the lead appeared in the flue gases of the multiple-hearth incinerator in Palo Alto, California. Dividing the total daily lead emission by the population served by the incinerator (250,000), Jacknow (155,156) calculated that the per capita discharge of lead was equivalent to driving an automobile using leaded gasoline with a lead concentration of 2 g/gal less than 200 feet per day (at an assumed 15 mpg rate).

Cadmium--Farrell et al. (154) showed that under some conditions, up to 30 percent of cadmium in sludge can escape as fine particulate matter. At the present time, there is no federal ambient air quality standard for cadmium and the public health implications of elevated cadmium levels associated with sludge incineration are not known.

Ash--Incineration is not an ultimate disposal process. About 10-30% of the total dry mass of sludge is converted to ash which is usually disposed in a landfill. Heavy metals which usually concentrate in sludge will also sometimes be concentrated in the ash. Heavy metals from the ash can leach into groundwater. The impacts of heavy metals in sludge ash in an actual disposal situation have not been studied yet.

Studies carried out by Dewling et al. (157) showed that Cd and Pb mostly remained in the ash (80% and 87%, respectively). They did not find evidence to suggest significant amounts of Pb or cadmium in the off-gas and particulates emitted from the incinerator.

Gray et al. (158) analyzed incinerator ash from seven municipalities. The ashes consisted mainly of oxides of silicon, aluminum, calcium and iron. All had basic pH (10.7 to 12.8) and a solubility of 1 to 2 percent dry weight of ash when leached with about 20 times their mass in water, indicating a low leachate potential in neutral or slightly alkaline groundwater.

Havens and Emerson (159) have conducted laboratory studies on the leachability of various trace metals in sludges before and after incineration using a standard citrate insolubility test to simulate the weak organic acid environment created by soil microorganisms. Compared to digested sludge, ash showed increased solubility of cadmium and copper, but decreased solubility of chromium, nickel and zinc. In both sludge and ash, lead showed no detectable solubility.

Pathogens--High temperatures used in incineration effectively destroy all pathogens in sewage sludge, leaving a sterile ash. Some incinerators use secondary effluent from wastewater treatment plants as scrubber water. Since gases passing through the scrubber water are at temperatures of 150 to 180°F, the temperature at which most viruses are destroyed, the possibility exists that a few viruses that may be present in the scrubber water might survive the 150 to 180°F temperature of stack gases and be transported out of the incinerator with particulate emissions. EPA tested stack gases of a single sewage sludge incinerator using a wet scrubber with chlorinated secondary effluent and found no viruses (152). However, it should be noted

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
PAGE AREA
FOR
DIMENSIONS
FOR 300 LB
AND 11 1/2
THICKNESS

that current methodology is not capable of routinely detecting airborne viruses even at wastewater treatment plants.

There may be a potential health hazard from exposure to pathogens in sludge to workers involved in the several dewatering processes which may precede sludge incineration.

Potential Health Effects of Incineration

In addition to the potential health hazard from exposure to pathogens in sludge to workers involved in the dewatering operations prior to incineration, another potential health risk from incineration is due to air pollution. As discussed in the previous section, the emission of heavy metals such as mercury, lead, cadmium, arsenic, etc. is not high enough to significantly alter the ambient air quality from sludge incineration (146). Incineration also nearly destroys pesticides and persistent organic chemicals present in sludge. Therefore health risks from organic chemicals due to sludge incineration would be negligible.

Other possible problems are emissions of oxides of nitrogen, particulates, and gases. Incinerators are not expected to be a significant source of atmospheric NO_x . The health impact of particulates and gaseous emissions should be negligible if incinerators are consistently operated such that the New Source Performance Standards are met. The potential health effects of air pollutants are listed in Table 90 (139,160).

Control of Air Pollution from Incineration

Procedures for curtailing emissions from sludge incinerators include reducing incineration temperature, as in fluidized bed burning, to decrease volatilization of metallic compounds; and installation of equipment to capture the emissions. To meet the new fly ash emission standards, new incinerators must employ high efficiency collectors such as electrostatic precipitators, wet scrubbers or fabric filters. Some states such as New York and California require afterburning, i.e., the addition of fuel to raise the final exhaust temperature to 1,400 to 1,500°F. This destroys odor-causing compounds and ensures more complete degradation of hydrocarbons, pesticides and polychlorinated biphenyls.

The New Source Performance Standards (NSPS) set by the USEPA limit particulate emissions from new sludge incinerators to no more than 70 mg/Nm³ (0.031 grains/standard cubic foot) and less than 20% opacity (145). Meeting the particulate performance standards requires a fly ash collector, because simple water sprays and baffle or settling chambers are not efficient enough to collect such fine dust. Accordingly, high-energy scrubbers, bag houses, or electrostatic precipitators are needed to achieve compliance with the standards.

The EPA Sewage Sludge Incineration Task Force (161) concluded that existing well-designed and properly operated municipal wastewater sludge incinerators have the capacity to meet the most stringent particulate emission control regulation existing in any state or local control agency. The

TABLE 90. HEALTH EFFECTS OF AIR POLLUTANTS (139,160)

Air quality level	Health effect	Pollutant levels			
		TSPa (24-hour), $\mu\text{g}/\text{m}^3$	SO ₂ (24-hour), $\mu\text{g}/\text{m}^3$	CO (8-hour), $\mu\text{g}/\text{m}^3$	NO ₂ (1-hour), $\mu\text{g}/\text{m}^3$
Significant harm		1,000	2,620	57,500	1,200 3,750
<u>General Health Effects</u>		Premature death of ill and elderly. Healthy people will experience adverse symptoms that affect their normal activity.			
<u>Precautions</u>		All persons should remain indoors, keeping windows and doors closed. All persons should minimize physical exertion and avoid traffic.			
Emergency	Hazardous	875	2,100	40,000	1,000 3,000
<u>General Health Effects</u>		Premature onset of certain diseases in addition to significant aggravation of symptoms and decreased exercise tolerance in healthy persons.			
<u>Precautions</u>		Elderly and persons with existing diseases should stay indoors and avoid physical exertion. General population should avoid outdoor activity.			
Warning	Very unhealthy	625	1,000	34,000	800 2,260
<u>General Health Effects</u>		Significant aggravation of symptoms and decreased exercise tolerance in persons with heart or lung disease, with widespread symptoms in the healthy population.			
<u>Precautions</u>		Elderly and persons with existing heart or lung disease should stay indoors and reduce physical activity.			

(continued)

TABLE 90 (continued)

Air quality level	Health effect	TSPa (24-hour), $\mu\text{g}/\text{m}^3$	SO ₂ (24-hour), $\mu\text{g}/\text{m}^3$	CO (8-hour), $\mu\text{g}/\text{m}^3$	O ₃ (1-hour), $\mu\text{g}/\text{m}^3$	NO ₂ (1-hour), $\mu\text{g}/\text{m}^3$
Alert	Unhealthful	375	800	17,000	400 ^b	1,130
<u>General Health Effects</u>		Mild aggravation of susceptible persons, with irritation symptoms in the health population.				
<u>Precautions</u>		Persons with existing heart or respiratory ailments should reduce physical exertion and outdoor activity.				
NAAQSC	Moderate	260	365	10,000	240	d
50 percent of NAAQSC	Good	75 ^e	80 ^e	5,000	120	d

^aTotal suspended particulates.

^b400 $\mu\text{g}/\text{m}^3$ was used instead of O₃ Alert Level of 200 $\mu\text{g}/\text{m}^3$.

^cNational Ambient Air Quality Standards.

^dNo index values reported at concentration levels below those specified by "Alert Level" criteria.

^eAnnual primary NAAQS.

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

newly promulgated federal New Source Performance Standards are based on demonstrated performance on an operating facility; thus, proper emission controls and proper operation of the incineration system must be used to meet all existing regulations of particulate matter.

Residue Disposal

The residues remaining after sludge combustion (ash, particulates from dry scrubbing, etc.) are typically placed in landfills. The ash from the combustion of municipal wastewater solids is free of pesticides, viruses and pathogens. If wastewater effluent is used as scrubber water, it may still contain low levels of viruses after use. The ash, however, generally contains high concentrations of trace metals in oxide form. Most of the heavy metal oxides are less soluble in water except for chromium compounds. Chromium will be converted to Cr^{+6} compounds in the incineration process which are soluble in water and may cause groundwater pollution from leaching of landfill ash. Leachate from sites where incinerator ash is landfilled should be controlled to prevent contamination of groundwater by metals. Many states are beginning to classify disposal sites according to their relationship to nearby groundwater and the material to be landfilled. Tables 91 and 92 describe methods used by the State of California for classifying waste materials and disposal sites (162). Typically, wastewater sludge incinerator ash requires a "protected" Class II-1 site and municipal refuse incinerator ash requires a hazardous fill site (139). These are described in Table 92.

Since the sludge ash concentrates the trace metals present in municipal sludge, recovery of these metals from the ash may be cost effective in certain special cases. A nationwide study of the concentrations of valuable metals in municipal sludges showed that the sludge from Palo Alto, California, contained some of the highest concentrations of gold, silver, copper and platinum of all the locations studied. The treatment plant in Palo Alto, receives the wastewater from many electronics firms. A private contractor, through a patented smelting process, has been reported to have extracted about a half-ounce of gold and 15-20 ounces of silver from each ton of sludge ash (163).

PYROLYSIS

Pyrolysis is a destructive distillation of sludge in the absence of oxygen at temperatures of about 800°F which are somewhat lower than those used in incineration (approximately 1400°F). In the pyrolysis process the organic portions of the sludge are converted into lower molecular weight compounds. The products of pyrolysis processes are combustible gases, tar and oil, and solid char. The gases generated in the pyrolysis process can be combusted for steam generation thus making it possible to burn sludge and other solid wastes with less excess air compared to incineration. This also results in lower gas volumes for cleaning and higher thermal efficiency.

Classical pyrolysis process is the destructive distillation, reduction or thermal cracking and condensation of organic matter under heat and/or pressure in the absence of oxygen. Complete absence of oxygen requires a

DROPPED
HEAD
BEGIN
SECTIONS
HERE

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

3/8"

205

PAGE NUMBER

TABLE 91. SOLID AND LIQUID WASTE CLASSIFICATION (139,162)

Group 1	Group 2	Group 3
Consist of or contain toxic substances and substances which could significantly impair the quality of usable waters. Examples include: • Saline fluids from water or waste treatment processes • Community incinerator ashes • Toxic chemical toilet waste • Industrial brines • Toxic and hazardous fluids • Pesticides or chemical fertilizers or their discarded containers • Other toxic wastes.	Consist of or contain chemically or biologically decomposable material which does not include toxic substances nor those capable of significantly impairing the quality of usable waters. Examples include: • Garbage • Rubbish • Construction debris such as paper, cardboard, rubber, etc. • Refuse such as yard, clippings, litter, glass, etc. • Dead animals • Abandoned vehicles • Sewage treatment residue such as solids from screenings and grit chambers, dewatered sludge and septic tank pumpings. • Infectious materials from hospitals or laboratories.	Consist entirely of nonwater soluble, nondecomposable inert solids. Examples include: • Construction and demolition debris, asphalt paving, inert plastics, etc. • Vehicle tires • Industrial wastes such as clay products, glass, slags, tailings, etc.

TABLE 92. CLASSIFICATION OF WASTE DISPOSAL SITES (139,162)

Class I	Class II	Class III
Class I disposal sites are those at which complete protection is provided for all time for the quality of ground and surface waters from all wastes deposited therein and against hazard to public health and wildlife resources. The following criteria must be met to qualify a site as Class I: (a) Geological conditions are naturally capable of preventing vertical hydraulic continuity between liquids and gases emanating from the waste in the site and usable surface or groundwaters. (b) Geological conditions are naturally capable of preventing lateral hydraulic continuity between liquids and gases emanating from wastes in the site and usable surface or groundwaters, or the disposal area has been modified to achieve such capability. (c) Underlying geological formations which contain rock fractures or fissures of questionable permeability must be permanently sealed to provide a competent barrier to the movement of liquids or gases from the disposal site to usable waters.	Class II disposal sites are those at which protection is provided to water quality from Group 2 and Group 3 wastes. The types of physical features and the extent of protection of groundwater quality divides Class II sites into the two following categories; Class II-1 sites are those overlying usable groundwater. Geologic conditions are either naturally capable of preventing lateral and vertical hydraulic continuity between liquids and gases emanating from the waste in the site and usable surface or groundwaters, or the disposal area has been modified to achieve such capability. Class II-2 sites are those having vertical and lateral hydraulic continuity with usable groundwater but for which geological and hydraulic features such as soil type, artificial barriers, depth to groundwater, and other factors will assure protection of the quality of usable groundwater underneath or adjacent to the site. The following criteria must be met to qualify a site as Class II;	Class III disposal sites are those at which protection is provided to water quality from Group 3 wastes by location, construction, and operation which prevent erosion of deposited material.

(continued)

TABLE 92 (continued)

Class I		Class II
(d)	Inundation of disposal areas shall not occur until the site is closed in accordance with requirements of the regional board.	(a) Disposal areas shall be protected by natural or artificial features so as to assure protection from any washout and from inundation which could occur as a result of tides or floods having a predicted frequency of once in 100 years.
(e)	Disposal areas shall not be subject to washout.	
(f)	Leachate and subsurface flow into the disposal area shall be contained within the site unless other disposition is made in accordance with requirements of the regional board.	(b) Surface drainage from the tributary areas shall not contact Group 2 waters in the site during disposal operations and for active life of the site.
(g)	Sites shall not be located over zones of active faulting or where other forms of geological change would impair the competence of natural features or artificial barriers which prevent continuity with usable waters.	(c) Gases and leachate emanating from waste in the site shall not unreasonably affect groundwater during the active life of the site.
(h)	Sites made suitable for use by man-made physical barriers shall not be located where improper operation or maintenance of such structures could permit the waste, leachate, or gases to contact usable ground or surface water.	(d) Subsurface flow into the site and the depth at which water soluble materials are placed shall be controlled during construction and operation of the site to minimize leachate production and assure that the Group 2 waste material will be above the highest and anticipated elevation of the capillary fringe of the groundwater. Discharge from the site shall be subject to waste discharge requirements.
(i)	Sites which comply with a, b, c, e, f, g, and h, but would be subject to inundation by a tide or a flood of greater than 100-year frequency may be considered by the regional board as a limited Class I disposal site.	

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTION
HERE

sealed chamber with external indirect firing and special construction materials which make the process expensive and inefficient. Partial pyrolysis or starved-air combustion is incomplete combustion and occurs when sufficient oxygen is provided to satisfy the combustion requirements. More conventional equipment can be used in this process. A number of different reactor types can be used for pyrolysis. There are two basic types, direct fired and indirect fired. In an indirect fired reactor, the feed stock materials are contained in an enclosed vessel and do not come in direct contact with the heating gases. The retort furnace and indirect fired rotary kiln are examples of this type of reactor. In a direct fired reactor a fraction of the stoichiometric oxygen required by the waste is admitted directly into the fuel fed to liberate the heat required for pyrolysis or gasification. Alternatively, hot combustion gases may be admitted to the reactor to heat the feed stock materials. Almost any incinerator reactor can be operated in a direct fired pyrolysis mode by simply reducing the air supply to less than the stoichiometric requirement. A multiple hearth furnace is an example of a reactor which can be operated in a combustion or direct fired pyrolytic mode. Most of the research and development in the field of pyrolysis of waste has been directed toward processing of municipal solid waste or copyrolysis of municipal solid waste and municipal sewage sludge.

At present there are very few pyrolysis projects under development in the United States that use municipal sludge alone. Several large scale projects have been proposed and some are in early operation, most work to 1979 has been on laboratory scale (139). Consequently, there is not very much information available on health impacts of pyrolysis of municipal sludge. Starved-air combustion, a partial pyrolysis process, had had a number of successful tests, such as those conducted at the Central Contra Costa Sanitary District (164,165), and several modular combustion units have used municipal solid waste, sewage sludge, and/or agricultural wastes (166).

Copyrolysis of sewage sludge and municipal solid waste in multiple hearth furnaces was recommended to the Central Contra Costa Sanitary District in California, by Brown and Caldwell (164,165). Brown and Caldwell Consulting Engineers conducted pilot tests in a multiple hearth furnace located in Concord, California, which was once used for sewage sludge incineration and had been shut down because of high energy costs. The test studies showed that pyrolysis was easier to control than incineration. The Concord project also used an afterburner at temperatures in excess of 1400°F with excess pyrolysis gas. This would result in almost complete combustion of organic chemicals.

The Orange County Sanitation Districts, California, have operated a pilot pyrolysis plant using the Activated Carbon Treatment System (ACTS) developed by the Jet Propulsion Laboratory, Pasadena, California. In this process, dewatered raw sludge was converted into a form of activated carbon.

Copyrolysis of municipal refuse and dewatered sewage sludge was carried out in a test program using a PUROX system developed by Union Carbide Corporation in South Charleston, West Virginia (167). The PUROX system consists of an oxygen-fed shaft furnace in which the solid waste is dried, pyrolyzed, combusted, and the inorganic residues slagged.

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLE
AND ILLU-
STRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD,
BEGIN
SECTIONS
HERE

Arlington County Water Pollution Control Plant, Virginia, is considering the use of a multiple hearth incinerator/pyrolyzer combination unit for the disposal of sewage sludge (168). The pyrolysis system is scheduled to be completed in 1980.

Starved-air combustion has also had some failures such as the demonstration plant in Baltimore, Maryland, which used only municipal solid waste (139,169). Baltimore used the Monsanto-Landgard process. It was a direct fired rotary kiln reactor and was used to pyrolyze shredded municipal refuse. The furnace in Baltimore, Maryland, is now being modified for further testing and use (139).

Air Pollution from Pyrolysis

The fact that pyrolysis operates without excess air and produces smaller volume of gas for cleaning than does incineration would indicate that the emissions from pyrolysis would be much lower than that from incineration (170). The behavior of the harmful substances in the pyrolysis process in a pilot experiment were analyzed by Majima et al. (170). Their study showed that results comparable to that of incineration regarding weight reduction of sludge can be achieved when pyrolysis is allowed to proceed for an adequate time period. But they also found that it takes a relatively long period of time to terminate the gasification reaction at approximately 800°F because of its low reaction rate.

9-1/8"

Since the temperatures used in pyrolysis process are lower than those needed in incineration, the pyrolysis process may lead to incomplete combustion, leading to gaseous emissions, dust and polycyclic aromatic hydrocarbons. The pyrolysis process may fail to oxidize completely and thus destroy organics and pesticides. Afterburning, a form of fuel-supported gaseous incineration, would be required to assure thorough destruction of such substances.

Heavy Metals--

The vaporization of the heavy metals would be less than that in incineration, most metals would be concentrated in the char, and as such would not be of concern in emissions from pyrolysis process (171,172). The trace metal levels found in the particulates remaining in the product gas after cleaning with an electrostatic precipitator, in South Charleston, WVA, are shown in Table 93 (167). These levels are compared with time-weighted average (TWA) threshold limit values (TLVs) proposed by the American Conference of Governmental Industrial Hygienists (38). As can be seen from Table 93, heavy metal emissions from refuse only or from refuse and sludge operation were far lower than the threshold limit values (TLVs) established for occupational exposure. Mercury may volatilize at the temperatures used in pyrolysis and, therefore, should be removed by scrubbers.

In the pyrolysis process, Cr⁺³ will not be oxidized to Cr⁺⁶ since there is very little air or O₂. Cr⁺³ compounds are insoluble in water and, therefore, will not cause groundwater pollution from leaching of landfilled ash (173).

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA;
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUS-
TRATIONS

TABLE 93. TRACE METAL CONCENTRATIONS IN PRODUCT GAS
COMBUSTION PRODUCTS^a (167)
(mg/m³)

Metal	Refuse ^b	Refuse + Sludge ^{c,d}	TLV-TWA
Cd	0.0022	0.0001	0.05
Cr	0.0090	0.0007	0.5
Cu	0.079	0.0097	0.2
Fe	1.62	0.03	5
Hg	0.0084	below detection limit	0.05
Pb	0.038	0.002	0.15
Zn	0.047	0.009	5

^aComputed from metal content in isokinetically collected particulate in the product gas.

^bRefuse-only with particulate recycle and non-optimum gas cleaning.

^cBased on average of measurements using primary and mixed sludges with more optimum gas cleaning performance.

^dApproximately 5% sludge-dry-solids/refuse.

Particulates--

The dust particles generated in the pyrolysis process, in general, are larger than those from incineration and, therefore, can be removed more efficiently.

Toxic Organic Chemicals and Pesticides--

The lower temperatures employed in pyrolysis process compared to incineration would lead to incomplete combustion of organic chemicals and may cause air pollution. Appropriate control measures should be employed for the control of such emissions.

Pathogens--

Complete destruction is possible at the temperatures employed in pyrolysis process.

CONCLUSIONS AND RECOMMENDATIONS

Incineration is one of the sludge disposal methods which is capable of completely destroying pathogens and persistent organic chemicals. Incineration, however, can lead to air pollution because elements such as sulfur, nitrogen and some volatile metals like mercury and arsenic are converted to gaseous compounds.

The degree of public health hazard caused by the air pollutants generated by sludge incinerators will be site specific and will depend on the nature of emissions, local meteorological conditions, ambient air quality levels, emissions from other sources, and geography of the region. The amounts of sulfur and nitrogen oxides produced by sludge incinerators, however, are usually small and therefore will only have a minor impact on ambient air quality. Also, the air pollution can be reduced or eliminated by using appropriate control measures. It is therefore concluded that sludge can be successfully incinerated if the incinerators are properly operated.

At present there are very few pyrolysis projects under development that use municipal sludge alone in the United States. Several large scale projects have been proposed and some are in early operation, most work to 1979 has been on laboratory scale. Consequently, there is not very much information on health impacts of pyrolysis. However, since pyrolysis process uses lower temperatures than incineration and since pyrolysis process operates without excess air, the airborne emission problem from pyrolysis is believed to be generally less difficult to manage than that from incineration. This would be true of heavy metals but the organic chemicals in sludge may only undergo incomplete combustion at the temperatures used in the pyrolysis process producing emissions requiring appropriate control measures for successful operation. After burning, a form of fuel-supported gaseous incineration, if used, can assure complete combustion of such substances.

REFERENCES

1. Clark, C. S., E. J. Cleary, G. M. Schiff, C. C. Linnemann, Jr., J. P. Phair and T. M. Briggs. Disease Risks of Occupational Exposure to Sewage. J. Environ. Eng. Div. Am. Soc. Civil Engineers, 102(EE2): 375-388, 1976.
2. Clark, C. S. et al. Health Risks of Human Exposure to Wastewater. Final Report, U.S. Environmental Protection Agency, Health Effects Research Laboratory, Grant No. R 803643, Cincinnati, Ohio, 1980.
3. Millner, P. D., P. B. Marsh, R. B. Snowden and J. F. Parr. Occurrence of *Aspergillus fumigatus* During Composting of Sewage Sludge. Appl. Environ. Microbiol. 34(6):767-772, 1977.
4. Boswell, F. C. Municipal Sewage Sludge and Selected Element Applications to Soil: Effect on Soil and Fescue. J. Environ. Qual. 4: 267-272, 1975.
5. Furr, A. K., G. S. Stoewsand, C. A. Bache and D. J. Lisk. Study of Guinea Pigs Fed Swiss Chard Grown on Municipal Sludge Amended Soil: Multi-element Content of Tissues. Arch. Environ. Health 31:87-91, 1976.
6. Hinesly, T. D., R. L. Jones, J. J. Tyler and E. L. Ziegler. Soybean Yield Responses and Assimilation of Zn and Cd from Sewage Sludge Amended Soil. J. Water Poll. Con. Fed. 48:2137-2152, 1976.
7. Kinzell, J. H., P. R. Cheeke and R. W. Chen. Tissue Heavy Metal Accumulation, Pentobarbital Sleeping Times and Multigeneration Reproductive Performance of Rats Fed Activated Sewage Sludge. Nutr. Rep. Intern. 15:645-650, 1977.
8. Process Design Manual. Sludge Treatment and Disposal. Municipal Environmental Research Laboratory, U.S. Environmental Protection Agency Report, EPA-600/1-79-011, 1979.
9. Environmental Resources Company. Workshop on the Health and Legal Implications of Sewage Sludge Composting, Massachusetts, December 18-20, 1978.
10. Detroy, R. W. et al. Aflatoxin and Related Compounds. In: Microbial Toxins: A Comprehensive Treatise, Vol. VI, Fungal Toxins, A. Ciegler, S. Kadis and S. J. Aji, Eds., New York, Academic Press, Inc., 1971.

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
TEXT LINE
HERE

11. Rylander, R., K. Andersson, L. Belin, G. Berglund, R. Bergstrom, L. Hanson, M. Lundholm and I. Mattsby. Studies on Humans Exposed to Airborne Sewage Sludge. *Schweiz. Med. Wschr.* 107:182-184, 1977.
12. Mattsby, I. and R. Rylander. Clinical and Immunological Findings in Workers Exposed to Sewage Dust. *Jour. of Occup. Med.* 20(10):690-692, 1978.
13. Rylander, R., K. Andersson, C. Belin, G. Berglund, R. Bergstrom, C. Hanson, M. Lundholm and I. Mattsby. Sewage Workers Syndrome. *Lancet* 28:478-479, 1976.
14. Rylander, R. et al. Local and Systemic Immune Response to Cotton Dust Bacteria. Paper presented at the 3rd Special Session on Cotton Dust, The Beltwide Cotton Conference, Phoenix, Arizona, 1979.
15. Rylander, R. Exposure to Gram-Negative Bacteria and the Development of Byssinosis. Paper presented at the XIX International Congress on Occupational Health, Dubrounik, Yugoslavia, 1978.
16. McGuire, W. et al. Activation of Plasma Contact System in Inflammatory Lung Disease. *Federation Proceedings* 39:906, 1980.
17. Dutkiewicz, J. Exposure to Dust-Borne Bacteria in Agriculture. I. Environmental Studies. *Arch. Environ. Health* 33(5):250-259, 1978.
18. Dutkiewicz, J. Exposure to Dust-Borne Bacteria in Agriculture. II. Immunological Survey. *Arch. Environ. Health* 33(5):260-270, 1978.
19. Lundholm, B. and R. Rylander. Occupational Symptoms Among Compost Workers. *J. Occup. Med.* 22(4):256-257, 1980.
20. Millner, P. D., D. A. Bassett and P. B. Marsh. Dispersal of *Aspergillus fumigatus* from Sewage Sludge Compost Piles Subjected to Mechanical Agitation in Open Air. *Appl. Environ. Microbiol.* 39(5):1000-1009, 1980.
21. May, K. R. Calibration of a Modified Andersen Bacterial Aerosol Sampler. *Appl. Microbiol.* 12(1):37-43, 1964.
22. Passman, F. J. Energy Resources Co. Personal Communication, November 12, 1979.
23. Clark, C. S., H. S. Bjornson, J. W. Holland, T. L. Huges, V. A. Majeti and P. S. Gartside. Occupational Hazards Associated with Sludge Handling. Chapter 10 in G. Bitton, B. L. Damron, G. T. Edds and J. M. Davidson, Eds. *Sludge-Health Risks of Land Application*, Ann Arbor Science, Ann Arbor, Michigan, 1980.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
EXTERIOR
DIMENSIONS
FOR TABLES
AND FIGURES

24. Rippon, J. W. Medical Mycology: The Pathogenic Fungi and the Pathogenic Actinomycetes, W. B. Saunders Co., Philadelphia, Pennsylvania, 1974.
25. Westphal, O. and K. Jans. Bacterial Lipopolysaccharides. Extraction with Phenol-Water and Further Applications of the Procedures. In: Methods in Carbohydrate Chemistry, Vol. 5, R. L. Whistler and M. L. Wolfrom, Eds., New York, Academic Press, Inc., 1965, pp. 83-91.
26. Gewurz, H. and L. A. Sujehira. Complement. In: Manual of Clinical Immunology, N. R. Rose and H. Friedman, Eds., American Society for Microbiology, Washington, D.C., 1976, p. 36.
27. Engrall, E. and P. Perlmann. Enzyme-Linked Immunosorbent Assay. III. Quantitation of Specific Antibodies by Enzyme-Labeled Anti-Immunglobulin in Antigen Coated Tubes. J. Immunol. 109:129-134, 1972.
28. Coleman, R. M. and L. Kaufman. Use of the Immunodiffusion Test in the Serodiagnosis of Aspergillus. Appl. Microbiol. 23(2):301-308, 1972.
29. Mancini, G., A. D. Carbonera and J. F. Heremans. Immunochemical Quantitation of Antigens by Single Radial Immunodiffusion. Immunochem. 2:235-239, 1965.
30. APHA, AWWA, WPCF. Standard Methods for the Examination of Water and Wastewater. 14th Edition, American Public Health Association, Washington, D.C., 1976.
31. Conover, W. J. Practical Nonparametric Statistics, Wiley and Sons, New York, New York, 1971.
32. Rima, D. R., E. Brown, D. F. Goerlitz and L. M. Law. Potential Contamination of the Hydrologic Environment from the Pesticide Waste Dump in Hardeman County, Tennessee. Administrative Report of the U.S. Geological Survey to the Federal Water Pollution Control Administration, August, 1967.
33. Tennessee Stream Pollution Control Board. Transcript of Hearing on the Velsicol Chemical Company Waste Disposal Site in Hardeman County. Jackson, Tennessee, March 4, 1971.
34. Sprinkle, C. L. Leachate Migration from a Pesticide Disposal Site in Hardeman County, Tennessee. U.S. Geological Survey, Open File Report 78-128, Nashville, Tennessee, 1978.
35. Clark, C. S., V. A. Majeti and V. J. Elia. Urine Screening of Workers Exposed to Toxic Waste Chemicals in a Municipal Wastewater Treatment Plant. University of Cincinnati, Department of Environmental Health Annual Report, Cincinnati, Ohio, 1979, pp. 224-227.

36. U.S. Environmental Protection Agency, Region IV. Summary of USEPA and State of Tennessee Chemical Analysis. Atlanta, GA, March 9, 1979.
37. Keith, L. H. and W. A. Telliard. Priority Pollutants I - A Perspective View. Environ. Sci. Technol. 13(4):416-423, 1979.
38. Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1977. American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio, 1979.
39. Elia, V. J., C. S. Clark and V. A. Majeti. Evaluation of Worker Exposure to Organic Chemicals at Municipal Wastewater Treatment Facilities. University of Cincinnati, Department of Environmental Health Annual Report, Cincinnati, Ohio, 1979, pp. 228-232.
40. Robbins, B. H. The Absorption, Distribution, Excretion of Carbon Tetrachloride in Dogs Under Various Conditions. Jour. Pharmacol. 37:203, 1929.
41. Barrett, H. M., J. G. Cunningham and J. H. Johnson. Study of Fate in Organisms of Some Chlorinated Hydrocarbons. Jour. Ind. Hyg. Toxicol. 21:479, 1939.
42. U.S. Environmental Protection Agency. Preliminary Assessment of Suspected Carcinogens in Drinking Water. Office of Toxic Substances, U.S. EPA, Washington, D.C., 1975.
43. U.S. Environmental Protection Agency. Preliminary Assessment of Suspected Carcinogens in Drinking Water. Appendices. EPA 560/4-75/003A. U.S. Environmental Protection Agency, Washington, D.C., 1977.
44. U.S. Environmental Protection Agency. OWPS Criterion Document on CCl₄, 1978.
45. American Chemical Society. Carbon Tetrachloride Spill Causes Stir Along the Ohio River. Chem. Engg. News 55:7, February 28, 1977.
46. National Research Council. Nonfluorinated Halomethanes in the Environment. National Academy of Sciences, Washington, D.C., 1978.
47. Lillian, D. et al. Atmospheric Fates of Halogenated Compounds. Environ. Sci. & Technol. 9(12):1042-1048, 1975.
48. National Academy of Sciences. Chloroform, Carbon Tetrachloride and Other Halomethanes: An Environmental Assessment. Washington, D.C., 1978.
49. Louri, D. B. and J. D. Bogden. The Dangers from Limited Exposure to Carbon Tetrachloride. CRC Critical Reviews in Toxicology 7(2):177-188, 1980.

50. Ingle, L. The Toxicity of Chlordane Vapor. Science 118:213, 1953.
51. Carter, M. R. The Louisville Incident. Internal Report (unpublished). Surveillance and Analysis Division, Region IV. U.S. Environmental Protection Agency, Athens, GA, 1977.
52. National Institute for Occupational Safety and Health. Registry of Toxic Effects of Chemical Substances. 403, 1978.
53. Treon, T. F., F. P. Cleveland and J. Cappet. The Toxicity of Hexachlorocyclopentadiene. Indust. Health 11:459-472, 1955.
54. U.S. Environmental Protection Agency. Review of the Environmental Effects of Pollutants. XI. Hexachlorocyclopentadiene. EPA-600/1-78-047, Cincinnati, Ohio 1978.
55. U.S. Environmental Protection Agency. Hexachlorocyclopentadiene. Ambient Water Quality Criteria, Washington, D.C., 1979.
56. Spehar, R. L. et al. Toxicity and Bioaccumulation of Hexachlorocyclopentadiene, Hexachloronorborene and Heptachloronorborene in Larval and Early Fathead Minnows. Pimephales Promelas, Bull. Environ. Cont. Toxicol. 21:576-583, 1979.
57. Cody, T. E., V. J. Elia and C. S. Clark. Preliminary Toxicity and Mutagenicity Evaluation of Groundwater Contaminated by an Abandoned Landfall. University of Cincinnati, Department of Environmental Health Annual Report, Cincinnati, Ohio, 1979, pp. 202-207.
58. Simmon, V. F. Affidavit U.S. District Court, Western District of Tennessee, Western Division, U.S.A. vs. Velsicol Chemical Corporation, Civil Action No. C-78-2335, July 1978.
59. Rima, D. R. Susceptibility of the Memphis Water Supply to Contamination from the Pesticide Waste Disposal Site in Northeastern Hardeman County, Tennessee. U.S. Geological Survey, Open File Report 79-750, Nashville, Tennessee, 1979.
60. Mississippi-Arkansas-Tennessee Council of Governments, Memphis Delta Development District. Section 208 Areawide Waste Treatment Management Plan, Waste Treatment Facilities Plan, Vol. 1, Chapter 1, History of Wastewater Collection System Development and Existing Wastewater Discharge Information, March 4, 1977.
61. Environmental Management Planning and Engineering, Inc. Influent Hazard Analysis, Memphis North Sewage Treatment Plant, Memphis, Tennessee, August-September 1977.
62. Communication, August 30, 1977 from Acting Chief, Hazard Evaluation and Technical Assistance Branch, National Institute of Occupational Safety and Health to Mayor, City of Memphis, Tennessee.

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

ENTER
OF PAGE

TOP OF
MARGIN
AREA

CHOPPED
HEAD
BEGIN
SECTIONS
HERE

63. Morse, D. L., J. R. Kominsky, C. L. Wissemann, III and P. J. Landrigan. Occupational Exposure to Hexachlorocyclopentadiene: How Safe is Sewage? JAMA 241:2177-2179, 1979.
64. Kominsky, J. R., C. L. Wisemann, III and D. L. Morse. Hexachlorocyclopentadiene Contamination of a Municipal Wastewater Treatment Plant. Am. Ind. Hyg. Assoc. Jour. 41(8):552-556, 1980.
65. Elia, V. J., C. S. Clark, V. Majeti, T. Macdonald and N. Richdale. Worker Exposure to Organic Chemicals at an Activated Sludge Wastewater Treatment Plant. In: Proceedings of U.S. Environmental Protection Agency Symposium on Wastewater Aerosols and Disease, Cincinnati, Ohio, September 1979.
66. Majeti, V. A. and C. S. Clark. Health Risks of Organics in Land Application. J. Environ. Eng. Div. Am. Soc. Civil Engineers (accepted for publication, 1980).
67. Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1977. American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio, 1979.
68. Spehar, R. L. et al. Toxicity of Bioaccumulation of Hexachlorocyclopentadiene, Hexachloronorbornadiene and Heptachloronorbornene in Larval and Early Juvenile Fathead Minnows. Bull. Env. Cont. Toxicol. 21:576-583, 1979.
69. Thomas, T. C. and J. N. Seiben. Chromosorb 102, An Efficient Medium for Trapping Pesticides from Air. Bull. Environ. Cont. Toxicol. 12: 17-25, 1974.
70. Neumeister, C. E. and R. W. Kurimo. Determination of Hexachlorocyclopentadiene and Octachlorocyclopentene in Air. Presented at American Industrial Hygiene Conference, May 7-17, Los Angeles, CA.
71. U.S. Department of Health Education and Welfare: NIOSH Manual of Analytical Methods, Vol. 5. NIOSH, Cincinnati, Ohio, 1977.
72. Burgessar, A. J. and J. F. Colaruotolo. Extraction of Semi- and Non-volatile Chlorinated Organic Compounds from Water. Anal. Chem. 51: 1588-1589, 1979.
73. Ingle, L. The Toxicity of Chlordane Vapor. Science 118:213, 1953.
74. Treon, T. F., F. P. Cleveland and J. Cappet. The Toxicity of Hexachlorocyclopentadiene. Indust. Health 11:459-472, 1955.
75. Carter, M. R. The Louisville Incident. Internal Report (unpublished), Surveillance and Analysis Division, Region IV, U.S. Environmental Protection Agency, Athens, GA, 1977.

BEGIN
LAST LINE
OF TEXT

DO NOT
WRITE IN
MARGINS
ENTERED
DIMENSIONS
FOR TABLES
AND ILLUSTRATIONS

76. Mehendale, H. M. The Chemical Reactivity-Absorption Retention, Metabolism and Elimination of Hexachlorocyclopentadiene. Environ. Health Perspect. 21:275-278, 1977.
77. Demerjian, Y. A., D. R. Kendrick and T. R. Westman. Muskegon County Wastewater Management System: Report on Major Research Activities, 1972-1978. Muskegon County Department of Public Works, Muskegon.
78. The Muskegon County Plan of Wastewater Reuse. Public Works, October 1973.
79. Environmental Research Corporation. Biological Sampler/Collector XM2 Operator's Manual, December 18, 1974, St. Paul, MN.
80. Dahling, D. R. and R. S. Safferman. Survival of Enteric Viruses Under Natural Conditions in a Subarctic River. Appl. and Environ. Microbiol. 38(6):1103-1110, 1979.
81. Dulbecco, R. and M. Vogt. Plaque Formation and Isolations of Pure Lines with Poliomyelitis Viruses. J. Exp. Med. 99:167-182, 1954.
82. Hsiung, G. D. and J. L. Melnick. Plaque Formation with Poliomyelitis, Coxsackie and Orphan (ECHO) Viruses in Bottled Cultures of Monkey Epithelial Cells. Virology 1:533-535, 1955.
83. Rao, V. C., U. Chandorkar, N. U. Rao, P. Kumaran and S. B. Lakhe. A Simple Method for Concentrating and Detecting Viruses in Wastewater. Water Res. 6:1565-1576, 1972.
84. Safferman, R. S. and M. Morris. Assessment of Virus Removal by a Multi-Stage Activated Sludge Process. Water Res. 10:413-420, 1976.
85. Adams, M. H. Bacteriophages. Interscience Publishers, New York, 1959.
86. Kott, Y. Estimation of Low Numbers of Escherichia Coli Bacteriophage by Use of the Most Probable Number Method. Appl. Micro. 14(2):141-144, 1966.
87. Scarpino, P. V. Bacteriophage Indicators. In: Indicators of Viruses in Water and Food, G. Berg, Ed., Ann Arbor Science Publishers, Inc., Ann Arbor, MI, 1978.
88. Air Sampling Instruments for Evaluation of Atmospheric Contaminants. 4th Edition, American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio, 1972.
89. Andersen, A. A. New Sampler for the Collection, Sizing and Enumeration of Viable Airborne Particles. Jour. Bacteriol. 76(5): 471-488, 1958.

90. Fair, G. M. and W. F. Wells. Measurement of Atmospheric Pollution and Contamination by Sewage Treatment Works. Proceedings of 19th Annual Meeting New Jersey Sew. Works Assn., Trenton, N.J. 20, 1934.
91. Albrecht, C. R. Bacterial Air Pollution Associated with the Sewage Treatment Process. M.S. Thesis, University of Florida, Gainesville, August 1958.
92. Ladd, F. C. Airborne Bacteria from Liquid Waste Treatment Units. M.S. Thesis, Oklahoma State University, Stillwater, 1966.
93. Carnow, B., R. Northrop, R. Wadden, S. Rosenberg, J. Holden. A. Neal, L. Sheaff, P. Scheff and S. Meyer. Health Effects of Aerosols Emitted from an Activated Sludge Plant. EPA-600/1-79-019, U.S. Environmental Protection Agency, Cincinnati, Ohio, May 1979.
94. Johnson, D.E., D. E. Camann, H. J. Harding and C. A. Sorber. Environmental Monitoring of a Wastewater Treatment Plant. EPA-600/1-79-027, U.S. Environmental Protection Agency, Cincinnati, Ohio, August 1979.
95. Johnson, D. E., D. E. Camann, J. W. Register, R. E. Thomas, C. A. Sorber, M. N. Guentzel, J. M. Taylor and H. J. Harding. The Evaluation of Microbiological Aerosols Associated with the Application of Wastewater to Land: Pleasanton, California. EPA-600/1-80-015, U.S. Environmental Protection Agency, Cincinnati, Ohio, 1980, 191 pp.
96. Bausum, H. T., S. A. Schaub and C. A. Sorber. Viral and Bacterial Aerosols at a Wastewater Spray Irrigation Site. NSF/RA-761319, Presented at 76th Annual Meeting, American Society for Microbiology, Atlantic City, N.J., May 3, 1976, U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, MD.
97. Randall, C. W. and J. O. Ledbetter. Bacterial Air Pollution from Activated Sludge Units, Amer. Indus. Hyg. Assoc. Jour. 27:(6):506-519, November-December 1966.
98. Cronholm, L. S. Emission of Microbial Aerosols from Polluted Waters in Densely Populated Regions. Research Report No. 117, University of Kentucky, Lexington, KY, U.S. Department of the Interior, 1978.
99. Finch, J.E., J. Prince and M. Hawksworth. A Bacteriological Survey of the Domestic Environment. Microbiol. Lab. Pharmaceutical Div., Reckitt and Colman Ltd, Hull HU8 7DS, UK, J. Appl. Bacteriol. 45(3): 357-364, 1978.
100. Bagley, S. T., R. J. Seidler, H. W. Talbot, Jr. and J. E. Morrow. Isolation of Klebsiellae from Within Living Wood. Appl. Environ. Microbiol. 36(1):178-185, 1978.

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE

DROPPED
HEAD
BEGIN
SECTION
HERE

101. Knittel, M.D., R. J. Seidler, C. Eby and L. M. Cabe. Colonization of the Botanical Environment by Klebsiella Isolates of Pathogenic Origin. *Appl. Environ. Microbiol* 34(5):557-563, 1977.
102. Ducluzeau, R. S. Hudault and J. V. Galpin. Longevity of Various Bacterial Strains of Intestinal Origin in Gas Free Mineral Water. *Eur. J. Appl. Microbiol.* 3(3):227-236, 1976.
103. Gevaudan, M.-J., A. Blancard, C. Gulian and M.-N. Mallet. Resistance of *Pseudomonas Aeruginosa* and *Klebsiella Pneumoniae* in the Exterior Environment. *Mediterr. Med.* 3(74):13-16, 1975.
104. Varma, M. M., W. A. Thomas and C. Prasad. Resistance to Inorganic Salts and Antibiotics Among Sewage-Borne Enterobacteriaceae and Achromobacteriaceae. *J. Appl. Bacteriol.* 41(2):347-349, 1976.
105. Levi, J. A., S. C. Schimpff, R. G. Slawson and P. H. Wiernik. Evaluation of Radiotherapy for Localized Inflammatory Skin and Perianal Lesions in Adult Leukemia: A Prospectively Randomized Double Blind Study. *Cancer Treat. Rep.* 61(7):1301-1305, 1977.
106. Umenai, T., Y. Saitoh and M. Chiba. Septicemia Caused by Klebsiella oxytoca. *Tohku J. Exp. Med.* 124(4):393-394, 1978.
107. Gross, G. N., S. R. Rehm and A. K. Pierce. The Effect of Complement Depletion on Lung Clearance of Bacteria. *J. Clin. Invest.* 62(2): 373-378, 1978.
108. Harris, G. D., W. G. Johanson, Jr. and A. K. Pierce. Determinants of Lung Bacterial Clearance in Mice After Acute Hypoxia. *Am. Rev. Respir. Dis.* 116(4):671-677, 1977.
109. Flint, L. M., Jr., C. R. Voyles, J. D. Richardson and D. E. Fry. Missile Tract Infections After Transcolonic Gunshot Wounds. *Arch. Surg.* 113(6):727-728, 1978.
110. Baergaluppi, J. F., R. Negrom and H. M. de Severino. *Ann. Allergy* 42(2):95-98, 1979.
111. Mackay, D. and A. W. Wolkoff. Rate of Evaporation of Low-Solubility Contaminants from Water Bodies to Atmosphere. *Environ. Sci. Technol.* 7:611-614, 1973.
112. Dilling, W. L., N. B. Teforttiller and C. J. Kallos. Evaporation Rates and Reactivities of Methylene Chloride, Chloroform 1,1,1-Trichloroethane, Tetrachloroethene and Other Chlorinated Compounds in Dilute Aqueous Solutions. *Environ. Sci. Technol.* 9:833-873, 1975.
113. Thibodeaux, L. J. and J. D. Millican. Quantity and Relative Desorption Rates of Air-Strippable Organics in Industrial Wastewater. *Environ. Sci. Technol.* 11:879-883, 1977.

GROUP
LAST LINE
OF TEXT

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
1/4 PAGE

TOP OF
IMAGE
AREA

DROPPING
HEAD
BEGIN
SECTIONS
HERE

114. Maynard, J. E., D. W. Bradley, C. L. Hornbeck, R. M. Fields, I. L. Doto and F. B. Hollinger. Preliminary Serologic Studies of Antibody to Hepatitis A Virus in Populations in the United States. *J. Infect. Dis.* 134:528-530, 1976.
115. Hall, W. T., F. K. Mundon and D. L. Madden. Antibody to Hepatitis A in Mentally Retarded Inpatients. *The Lancet*, April 1977, pp. 78-9.
116. Rose, F. L. and J. C. Harshbarger. Neoplastic and Possibly Related Skin Lesions in Neotenic Tiger Salamanders from a Sewage Lagoon. *Science* 196:315-317, 1977.
117. Tipton, I. H. and M. J. Cook. Trace Elements in Human Tissue: Part II. Adult Subjects for the United States. *Health Physics* 9:103, 1963.
118. Kinzell, J. H., P. R. Cheeke and R. W. Chen. Tissue Heavy Metal Accumulation, Pentobarbital Sleeping Times and Multigeneration Reproductive Performance of Rats Fed Activated Sewage Sludge. *Nutr. Rep. Intern.* 15:645-650, 1977.
119. Odum, E. P., G. W. Barrett and H. R. Pulliam. Testing Pesticides at the Ecosystem Level. *ASB Bull.* 15:48, 1968.
120. Barrett, G. W. The Effects of an Acute Insecticide Stress on a Semi-Enclosed Grassland Ecosystem. *Ecology* 49:1019-1035, 1968.
121. Barrett, G. W., G. M. Van Dyne and E. P. Odum. Stress Ecology. *Bio-science* 26:192-194, 1976.
122. Odum, E. P. The Emergence of Ecology as a New Integrative Discipline. *Science* 195:1289-1293, 1977.
123. Woodwell, G. M. Recycling Sewage Through Plant Communities. *Amer. Sci.* 65:556-562, 1977.
124. Odum, E. P. the Strategy of Ecosystem Development. *Science* 164:262-270, 1969.
125. Sopper, W. E. and L. T. Kardos. Recycling Treated Municipal Wastewater and Sludge Through Forest and Cropland. Penn State University Press, University Park, PA, 1973, 494 pp.
126. Odum, E. P. Organic Production and Turnover in Old-Field Succession. *Ecology* 41:34-49, 1960.
127. Golley, F. B. Structure and function of an Old-Field Broomsedge Community. *Ecol. Manage.* 35:113-131, 1965.
128. Wiegert, R. G. and F. C. Evans. Primary Production and the Disappearance of Dead Vegetation on an Old-Field in Southeastern Michigan. *Ecology* 45:49-63, 1964.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TAB 22
AND ILLU-
STRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTIONS
HERE

129. Hurd, L. E., M. V. Mellinger, L. L. Wolf and s. J. McNaughton. Stability and Diversity at Three Trophic Levels in Terrestrial Successional Ecosystems. *Science* 173:1134-1136, 1971.
130. Krebs, C. J., B. L. Keller and r. H. Tamarin. Microtus Population Biology: Demographic Changes in Fluctuating Population of M. ochrogaster and M. pennsylvanicus in Southern Indiana. *EcoTogy* 50: 587-607, 1969.
131. Pitelka, F. A. and A. M. Schultz. The Nutrient-Recovery Hypothesis for Arctic Microtine Cycles. In: *Population Ecology*, L. Adams, Ed., Dickerson Pub. Co., Belmont, CA, 1970, 160 pp.
132. Taylor, W. G., G. W. Barrett and T. J. Anderson. The Effects of Sewage Sludge and Fertilizer on Primary Productivity and Diversity in an Annual and a Perennial Grassland Community. In review, *Amer. Midl. Nat.*
133. Carson, W. P., T. A. Boggs and G. W. Barrett. The Effects of Dried Sewage Sludge on a 1-Year and a 5-Year Old-Field. In preparation, *J. Environ. Mgmt.*
134. Sedlacek, J. D. and G. W. Barrett. Effects of Dried Sewage Sludge on Density and Diversity of Anthropol Communities in an Annual and a Perennial Grassland. In Preparation.
135. Anderson, T. J., G. W. Barrett, K. L. Stemmer, C. S. Clark, V. J. Elia, V. A. Majeti and T. E. Cody. Tissue Metal Concentration and Pathology in Meadow Voles from Sewage Sludge-Amended Fields. In preparation.
136. Anderson, T. J. and G. W. Barrett. Effects of Dried Sewage Sludge on Meadow Vole Populations in Two Grassland communities. In review, *J. Appl. Ecol.*
137. Burd, R. S. A Study of Sludge Handling and Disposal. Federal Water Pollution Control Research Service, Publication No. WP-20-4. Washington, D.C., Federal Water Pollution Control Administration, 1968.
138. National Academy of Sciences. Multimediu Management of Municipal Sludge. National Research Council. Analytical Studies of the U.S. Environmental Protection Agency, Volume IX, 1978.
139. U.S. Environmental Protection Agency. Sludge Treatment and Disposal. EPA-625/1-79-011, Cincinnati, Ohio, 1979. Chapter 11.
140. Russell, R. A. Theory of Combustion of Sludge. Sludge Concentration-Filtration and Incineration. School of Public Health, University of Michigan, Continued Education Series No. 113, Ann Arbor, Michigan, University of Michigan (out of print), 1964, pp. 152-157.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLU-
STRATIONS

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD;
BEGIN
SECTION
HERE

141. Shen, T. T. Air Pollution from Sewage Sludge Incineration. Proceedings of the American Society of Civil Engineers, Jour. of Env. Eng. Div. 105(EE1):61-74, 1979.
142. Furr, A. K. et al. Multielement and Chlorinated Hydrocarbon Analysis of Municipal Sewage Sludges of American Cities. Env. Sci. and Technol. 10(7):683-687, 1976.
143. Water Pollution Control Federation. Design of Wastewater Treatment Plants. Washington, D.C., Water Pollution Control Federation, 1977.
144. Federal Register 40(199):48292, October 14, 1975.
145. Devitt, T. W. and N. J. Kulujian. Sewage Sludge Incinerators-Inspection Manual for the Enforcement of New Source Performance Standards. EPA-340/1-75-004, U.S. Environmental Protection Agency, Washington, D.C., February 1975.
146. U.S. Environmental Protection Agency. Air Pollution Aspects of Sludge Incineration. Office of Technology Transfer, Seminar Publication, EPA-625/4-75-009, Washington, D.C., 1975.
147. U.S. Environmental Protection Agency. Land Treatment of Municipal Wastewater Effluents: Design Factor-I. EPA-625/4-76-010, Vol. 1, January 1976, PB-259955.
148. Gabler, R. C., Jr. and D. L. Neyland. A Study of Pesticide Disposal in a Sewage Sludge Incinerator. Versar, Inc., Monthly Progress Report, EPA Contract No. 68-01-1587, U.S. Environmental Protection Agency, Washington, D.C., September 1974, PB-253 485/7BE.
149. U.S. Environmental Protection Agency. Background Information on National Emission Standards for Hazardous Air Pollutants - Proposed Amendments for Asbestos and Mercury. EPA-450/2-74-0099, 1974a.
150. Whitmore, F. C. and R. J. Durfee. Lead and Mercury Balance at the Palo Alto Incinerator. Versar, Inc., 1974.
151. Los Angeles/Orange County Metropolitan Area (LA/OMA). Sludge Processing and Disposal - A State-of-the-Art Review. Regional Wastewater Solids Management Programs, California, 1977.
152. Workshop on the Health and Legal Implications of Sewage Sludge Composting. Vol. 2, Chapter 9, Environmental Resources company, December 18-20, 1978, Cambridge, Massachusetts.
153. Soldano, B. A., P. Bien and P. Kwan. Airborne Organo Mercury and Elemental Mercury Emissions with Emphasis on Central Sewage Facilities. Atmospheric Environment 9:941-944, 1975.

BEGIN
LAST LINE
OF TEXT

BOTTOM OF
IMAGE AREA
OUTSIDE
DIMENSION
FOR TABLES
AND ILLUSTRATIONS

154. Farrell, J. B., H. O. Wall and b. A. Kerdolff. Air Pollution from Sewage Sludge Incinerators. Unpublished Report. U.S. Environmental Protection Agency, Cincinnati, Ohio 1978.
155. Jacknow, J. Environmental Aspects of Municipal Sludge Incineration. In: Acceptable Sludge Disposal Techniques: Cost, Benefit, Risk, Health, and Public Acceptance, 47-51, Information Transfer, Inc., Rockville, Maryland, 1978,
156. Practical, Available Technology (PAT) Report. Sludge Incineration. Env. Sci. and Technol.10(12):1080-82, 1976.
157. Dewling, R. T., R. N. Manganelli and G. T. Baer, Jr. Fate and Behavior of Selected Heavy Metals in Incinerated Sludge. Jour. WPCF 52(10):2552-2557, 1980.
158. Gray, D. H. and C. Penessis. Engineering Properties of Sludge Ash. Jour. WPCF 44(5):847-858, 1972.
159. Havens and Emerson. Proposed Sludge Management Plan. Metropolitan District Commission, Boston, 1973.
160. U.S. Environmental Protection Agency. Environmental News, August 23, 1976.
161. U.S. Environmental Protection Agency. Sewage Sludge Incineration. Report of the Task Force for the Office of Research and Monitoring. EPA-R2-72-040, Washington, D.C., U.S. Environmental Protection Agency, PB-211 323, Springfield, Virginia, National Technical Information Service, 1972.
162. California Administrative Code: Title 23. Chapter 3, State Water Resources Control Board, Subchapter 15, Waste Disposal to Land, March 10, 1972 (updated on June 7, 1980).
163. Palo Alto Finds Gold in its Sludge Ash. Water Pollution Control Fed. Highlights 17(2):2, 1980.
164. Brown and Caldwell Consulting Engineers. Solid Waste Resource Recovery Full Scale Test Report. Central Contra Costa Sanitary District, California, Volumes One and Two, March 1977.
165. Sieger, R. B. and B. D. Bracken. Sludge, Garbage May Fuel California Sewage Plant. The American City and County, p. 37, January 1977.
166. Huffman, G. L. and W. W. Liberick, Jr. EPA's R & D Program in Pyrolytic Conversion of Waste to Fuel Products. In: Solid Wastes and Residues. Conversion by Advanced Thermal Processes. J. L. Jones and S. B. Radding, Eds., ACS Symposium Series 76, American Chemical Society, 1978.

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

ONLY TWO
HEAD
REGIONS
SECTIONS
HERE

167. Moses, C. T., K. W. Young, G. Stern and J. B. Farrel. Co-Disposal of Sludge and Refuse in a Purox Converter. In: Solid Wastes and Residues. J. L. Jones and S. B. Radding, Eds., ACS Symposium Series 76, American Chemical Society, 1978.
168. Galandak, J. and M. Racstain. Design Considerations for Pyrolysis of Municipal Sludge. Jour. WPCF 51(2):370-376, 1980.
169. Metz, W. C., J. Shyer and K. Edgecomb. Preliminary Assessment of the Prospects for Use of Refuse - Derived Fuel in Maryland. Brookhaven National Lab., Upton, N.Y., February 1979, 83 pp.
170. Mazima, T., K. Tadao, M. Naruse and M. Hiraoka. Studies on the Pyrolysis Process of Sewage Sludge. Prog. Water Tech. 9:381-396, 1977.
171. Olexsey, R. A. Pyrolysis of Sewage Sludge. Proceedings of the 1975 National Conference on Municipal Sludge Management and Disposal, Anaheim, California, August 18-20, 1975.
172. Lewis, F. M. Sludge Pyrolysis for Energy Recovery and Pollution Control. Proceedings of the Second National Conference on Municipal Sludge Management and Disposal. Anaheim, California, August 18-20, 1975.
173. U. S. Environmental Protection Agency. Proceedings of Fifth United States/Japan Conference on Sewage Treatment Technology, Chapter 4, EPA-600/9-77-027, April 18-22, 1977, Tokyo, Japan.

ENTER
LAST LINE
OF TEXT

ON THE
IMAGE AREA
OUTSIDE
OF MARGIN
FOR NAME OF
PAGE, ETC.

BEGIN
FIRST
LINE OF
TEXT
HERE

TYPING GUIDE SHEET

CENTER
OF PAGE

TOP OF
IMAGE
AREA

DROPPED
HEAD
BEGIN
SECTION
HERE

APPENDIX A

Muskegon

6/4/79

Date _____

Monthly Illness History

Name _____ I.D. Number _____

1. Have you been sick in any way in the past month? Yes _____ No _____

If "yes" then answer the following:

a. Briefly describe the illness, when it occurred, and how many days it lasted:

b. Indicate which of the following you had:

	Yes	No	# days
1. Fever (list highest recorded temperature)	_____	_____	_____
2. Chills	_____	_____	_____
3. Eye inflammation (red eye)	_____	_____	_____
4. Runny nose	_____	_____	_____
5. Sneezing	_____	_____	_____
6. Sore throat	_____	_____	_____
7. Coughing	_____	_____	_____
8. Shortness of breath	_____	_____	_____
9. Chest pain	_____	_____	_____
10. Pneumonia	_____	_____	_____
11. Nausea	_____	_____	_____
12. Vomiting	_____	_____	_____
13. Stomach pain	_____	_____	_____
14. Diarrhea	_____	_____	_____
15. Dermatitis (skin rash or infection)	_____	_____	_____

BEGIN
LAST LINE
OF TEXT

NOTICE OF
IMAGE AREA
EXTENDING
BEYOND THE
PAGE LIMITS
OF THE
DOCUMENT

